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Electrical Evidence for Dual Innervation of Muscle Fibers in the Sipunculid Golfingia (= Phascolosoma)[1]

C. LADD PROSSER AND NICK SPERELAKIS[2]
Marine Biological Laboratory and University of Illinois[3]

The short-fibered, non-striated proboscis retractor of *Golfingia* (= *Phascolosoma*) *gouldii* when stimulated directly shows two kinds of action potential: (1) a "fast" response which is propagated at about 1.3 m/second, fatigues rapidly on repetition and is associated with a phasic twitch, and (2) a "slow" response which is propagated at 0.3 m/second, facilitates on repetition and is associated with tonic contraction (Prosser, Curtis and Travis, '51). That both types of response are conducted in nerve fibers is shown by (1) abundance of nerve fibers of two sizes running longitudinally in the muscle, (2) absence of propagated response after nerves have degenerated, (3) block of conduction by low concentrations of procaine and tetracaine, (4) presence of nerve impulses ahead of the recorded muscle potentials and at the velocity of the muscle response, (5) local block of neuromuscular transmission (but not of the nerve impulses) by D-tubocurarine, and (6) potentiation of the slow response by physostigmine (Prosser and Melton, '54). It was concluded that there is no conduction between muscle fibers, that conduction in the phascolosoma proboscis retractor is entirely by nerve fibers and that the slow junctions are cholinergic. Since all of the muscle fibers appear similar histologically, it is of interest to learn whether individual muscle fibers can give both fast and slow responses, that is, whether they are dually innervated.

MATERIALS AND METHODS

The possibility of double innervation was investigated by recording electrical responses from individual fibers by means of microelectrodes of less than 0.5 µ tips, filled with 3 M KCl. A silver-silver chloride electrode led to a high input-impedance electrometer preamplifier. Auxiliary equipment such as calibrator, bucking voltages, DC output amplifier, oscilloscope and camera was conventional for high-impedance microelectrode recording. Electrodes were pulled by a mechanical puller; short- and medium-tapered tips were preferable to long ones. Electrodes were screened microscopically and by measurements of resistance and tip potential before use. The preparation of the muscle has been described previously (Prosser, Curtis and Travis, '51). The muscle was threaded over and below successive glass rods in a bath of sea water in order to minimize movement during contraction and thus prevent excessive breakage of the microelectrodes. Sometimes the muscles were treated for a few minutes with chymotrypsin in sea water; this softened the sheath and made penetration easier without altering potentials. Microelectrodes were usually positioned under a dissecting microscope by a hydraulic micromanipulator. In some of the later experiments floating microelectrodes held on chlorided silver coils were used.

RESULTS

Penetration of the fibers of the proboscis retractor by microelectrodes proved to be difficult and a wide range of apparent resting potentials was obtained. Criteria of contact with the interior of a fiber were: sudden drop to a negative potential which held for a reasonable period, rapid rise to zero on withdrawal or further penetration, action potentials of opposite polarity to those recorded outside the fiber.

---

[1] The generic name of the common Cape Cod sipunculid which has been *Phascolosoma* in previous experimental papers in now *Golfingia*. (Fisher, W. K. '50, '52). It is here proposed to use phascolosoma as a common name.

[2] Present address, Physiology Department, Western Reserve University, Cleveland, Ohio. Help from Dr. W. W. Steinberger in some of the later experiments (1958) is gratefully acknowledged.

[3] Supported in part by NSF and NIH contracts. Observations were made during summers of 1956, 1957 and 1958.

Intracellularly recorded resting potentials were low, compared with many other kinds of muscle; 90 determinations (summer of 1957) averaged 25 mv with a range from 11 to 49 mv. The equilibrium potential calculated from the potassium gradient (Steinbach, personal communication) is about 73 mv. The true membrane poten-

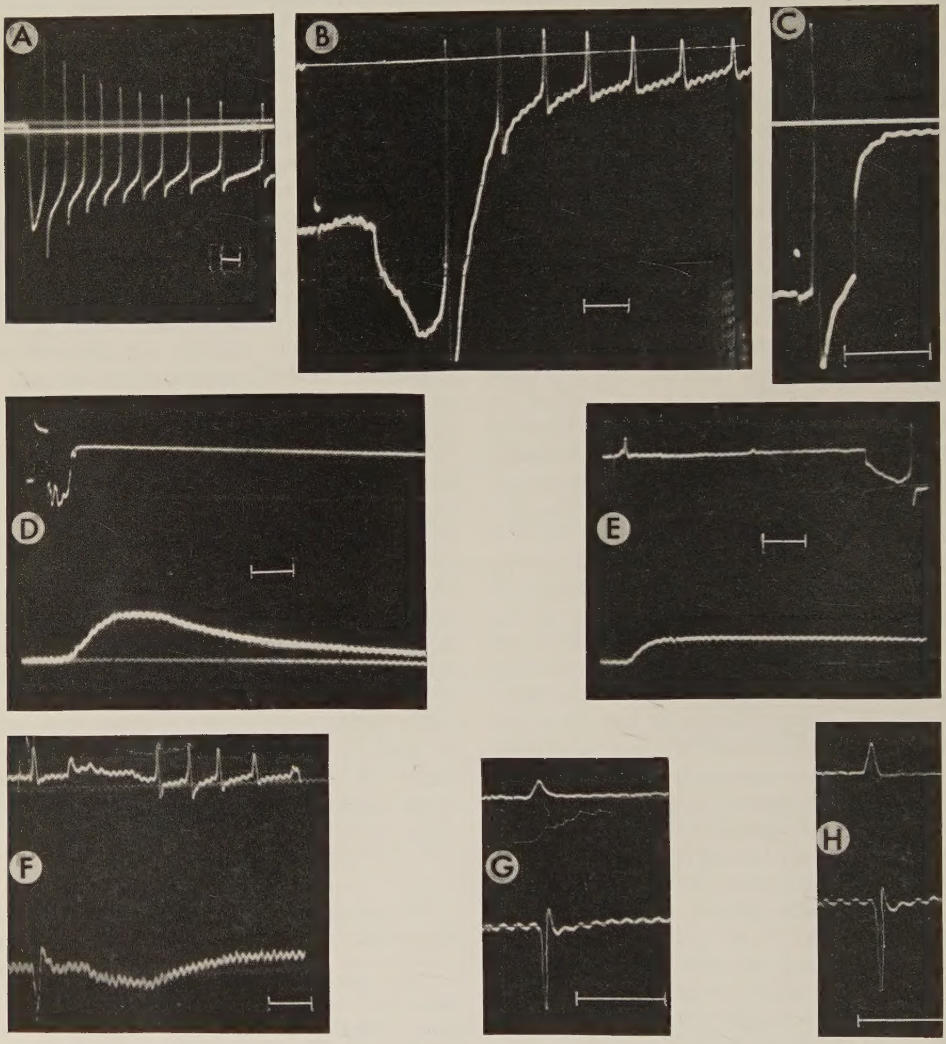

Fig. 1 Action potentials recorded by microelectrodes from proboscis retractor of *Golfingia*. Time marks 0.1 second A,B,D, "injury" spikes, A on penetration of electrodes, B on exit, and D after stimulation, resting potential in B 32 mv (zero potential line extrapolated in B); D, E, potentials on upper beam, contractions on lower beam, in D a fast response followed by withdrawal, in E a slow response with an injury spike at end of sweep; F,G,H, intracellular response on upper beam, extracellular on lower to show opposite polarity; F intracellular (upper) and extracellular (lower) records show initial response, only intracellularly the injury spikes.

tial cannot be predicted in the absence of permeability values for K^+, Na^+ and Cl^-. A few measurements have been made with the sucrose gap electrode and isotonic K_2SO_4 (Burnstock and Prosser, unpublished); these averaged 52 mv. The low values could not be attributed to the microelectrodes because the same electrodes yielded high potentials in frog sartorius or dogfish smooth muscle. High resistance (30 megohms) electrodes tended to give higher resting potentials than 10-megohm electrodes. Frequently on penetration there was an initial small drop, then a larger one presumably as electrode seal improved. Also as the microelectrode entered or left a fiber there was often injury as evidenced by trains of spikes (fig. 1 A.B.C). It was usually impossible to keep a microelectrode, even of the floating type, inserted for more than a fraction of a minute in a muscle fiber. Any contraction pushed the electrode out and withdrawal usually followed an observed

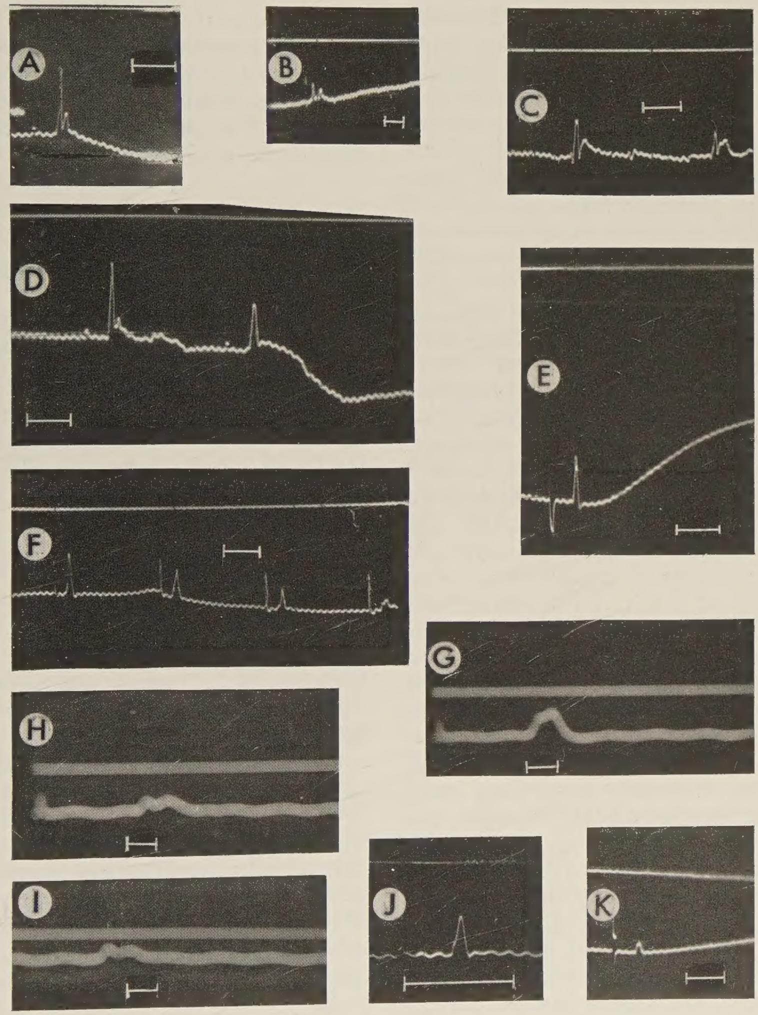

Fig. 2 Intracellular potentials. Upper beam in all records is zero potential and level of lower beam corresponds to recorded resting potential. All time marks 0.1 second; A,B,C fast and slow responses; D,E,F, fast responses only. Resting potential in E 40 mv; J,K, slow responses only; G,H,I, successive fast and slow responses as electrode gradually withdrew from fiber, initial R.P. in G 9 mv.

electrical response. When a muscle was first mounted, it was highly excitable and sometimes showed local contractions; at this time resting potentials were low. After some time excitability declined, recorded resting potentials were often larger (40 mv), and potentials were then maintained for several minutes. It is probable that the observed values depend partly on the seal of the cell membrane around the electrode. A poor seal results in much shunting, hence low apparent resting potentials. Not much reliance is placed on the absolute values of the recorded resting potentials, particularly the lower ones. However, there is no doubt that contact was made with the cell interiors and that the question of dual innervation can be settled, even with low resting potential values.

When a proboscis retractor was stimulated at some distance from an internal microelectrode, responses representing depolarization were observed. These action potentials were in opposite polarity to responses recorded simultaneously with external electrodes (fig. 1 G,H). They were also correlated with contractions (fig. 1 D,E). Some of the intracellular responses were clearly fast spikes; these declined on repetition as do fast responses obtained with external recording (fig. 2 D,E,F). Many fibers gave both fast and slow responses and the slow ones facilitated (fig. 2 A,B,C,G). Still other fibers gave responses with the time relations of slow potentials (fig. 2 J,K). Evidently, therefore, many fibers have a double innervation, fast and slow; but some are innervated by only one kind of nerve fiber. The proportion of fibers receiving slow innervation is difficult to ascertain since slow responses are very small. However, nearly half the fiber responses were fast only, slightly fewer combined fast and slow, and a few were purely slow.

Neither the fast nor slow responses ever reached zero potential, i.e., they failed to show overshoot of potential. By this criterion they may be considered as similar to end-plate potentials. As the recorded membrane potential decreased during partial withdrawal the responses declined (fig. 2 G,H,I).

Frequently on entry or exit of the microelectrode or following electrical stimulation large spikes were seen, sometimes in trains of decreasing amplitude and frequency (fig. 1 A,B,C). The initial spikes often showed an overshoot and were followed by hyperpolarization. The spikes were not recorded by external wick electrodes, hence were restricted to the impaled fiber (fig. 1 F). These responses are all-or-none type spikes presumably due to injury to the membrane. Attempts to stimulate and record with the same intracellular electrode were unsuccessful.

It is concluded that many but not all of the fibers in the proboscis retractor receive double innervation and that the responses, both fast and slow, are of the graded junction type potential although the cell membranes are capable of spike-type depolarizations with overshoots.

DISCUSSION

The *Golfingia* proboscis retractor is a relatively fast muscle and in it conduction is entirely by nerves (Prosser and Melton, '54). The correspondence of the graded fast and slow potentials with those detected externally and the restriction of spikes to the impaled fiber is further evidence against conduction in the muscle. Similar trains of spikes are seen during penetration into some nerves cells (Alanis and Matthews, '52).

The preceding evidence indicates that many of the individual fibers, though small in size, have a double innervation. Double (or multiple) innervation has been demonstrated in striated muscles of insects (Hoyle, '55), crustaceans (Wiersma, '53), intrafusal fibers of amphibians (Eyzaguirre, '57) and it is indicated for nonstriated muscles of some molluscs (Prosser, unpublished) and coelenterates (Horridge, '56). It is probable that dual innervation of muscle fibers is more widespread than the single innervation known for fast striated muscle fibers of higher vertebrates. Whether individual smooth muscle fibers of mammals receive both parasympathetic and sympathetic innervation is not known.

In crustacean muscles with multiple innervation some fibers receive fast, some slow and some both fast and slow innervation (Furshpan from Wiersma, '57; Hoyle, '57). Similarly the muscle fibers of the proboscis retractor do not all have the same innervation.

The responses, both fast and slow, of the *Golfingia* muscle are of the junction type (post-synaptic potentials, p.s.p.'s) although the membrane is capable of overshooting spike-type potentials. In this respect also this muscle resembles crustacean muscle in which normal fast and slow responses are p.s.p.'s but propagated spikes can occur in response to transmembrane stimulation (Fatt and Katz, '53).

SUMMARY

Microelectrodes made contact with muscle fiber interiors as evidenced by sudden negativity on penetration and by responses in reverse polarity to those recorded externally. Recorded resting potentials were low (average 25 mv).

In response to shocks some fibers gave fast responses only, many gave both fast and slow responses and a few only slow potentials. Responses were only partial depolarizations and appear to be post-synaptic type potentials (p.s.p.'s).

Associated with penetration or withdrawal, overshooting spike-type potentials were recorded, often in trains of decreasing amplitude and frequency.

It is concluded that the non-striated short muscle fibers of the *Golfingia* proboscis retractor resemble crustacean muscle in that many fibers have dual motor innervation, yield endplate-type potential responses and are capable of spikes.

LITERATURE CITED

Alanis, J., and B. H. C. Matthews 1952 The mechano-receptor properties of central neurones. J. Physiol., *117:* 59P–60P.

Eyzaguirre, C. 1957 Functional organization of neuromuscular spindle in toad. J. Neurophysiol., *20:* 523–542.

Fatt, P., and B. Katz 1953 The electrical properties of crustacean muscle fibers. J. Physiol., *120:* 171–204.

Fisher, W. K. 1950 The genus *Phascolosoma.* Annals and Mag. Nat. Hist., 3, Ser., *12:* 547–552.

——— 1952 Sipunculid worms of California and Baja California. Proc. U. S. Nat., Mus., *102:* 371–450.

Furshpan, E. Thesis cited in Wiersma, C. A. G. 1957 Neuromuscular mechanisms. In: Rec. Adv. Invert. Physiol., B. Scheer, ed., Univ. Oregon, Eugene, Ore., pp. 143–159.

Horridge, G. A., 1956 The nerves and muscles of medusae. V. Double innervation in Scyphozoa. J. Exp. Biol., *33:* 366–383.

Hoyle, G. 1955 The anatomy and innervation of locust skeletal muscle. Proc. Roy. Soc. Lond. Ser. B., *143:* 281–291.

Hoyle, G. 1957 Comparative physiology of nervous control of muscular contraction. Camb. Univ. Press., Cambridge, 147 pp.

Prosser, C. L., H. J. Curtis and D. M. Travis 1951 Action potentials from some invertebrate non-striated muscles. J. Cell. and Comp. Physiol., *38:* 299–320.

Prosser, C. L., and C. E. Melton 1954 Nervous conduction in smooth muscle of *Phascolosoma* proboscis retractors. Ibid., *44:* 255–276.

Wiersma, C. A. G. 1953 Neural transmission in invertebrates. Physiol. Rev., *33:* 326–355.

Responses to Stretch and the Effect of Pull on Propagation in Non-Striated Muscles of Golfingia (= Phascolosoma) and Mustelus

C. LADD PROSSER, C. L. RALPH AND WM. W. STEINBERGER
Marine Biological Laboratory, University of Illinois, U. S. Department of Agriculture, and University of Michigan[1,2]

In the short-fibered non-striated proboscis retractor of phascolosoma (*Golfingia gouldii*),[3] conduction is entirely by intrinsic nerve fibers. Evidence for nerve conduction is: velocity of propagation (1.3 and 0.3 m/second for fast and slow responses, respectively), abundance of nerve fibers, block by nerve-blocking drugs, failure of propagation after nerve degeneration, and detection of nerve impulses ahead of muscle responses (Prosser and Melton, '54). Conduction in other non-striated postural muscles of invertebrates appears also to be by nerves. In vertebrate visceral smooth muscle, in contrast, conduction is from fiber to fiber independently of nerves and is very slow (few centimeters per second) (Bozler, '48, Prosser and Sperelakis, '56). In the circular intestinal muscle of the cat, conduction can pass a region clamped to prevent mechanical pull, it can occur under such tension that shortening is prevented, and mechanical and electrical responses can be partly dissociated by drugs (Sperelakis and Prosser, '59); hence mechanical pull appears unnecessary for muscle activation. However, some smooth muscles are stimulated by stretch, even in the absence of nerves and myenteric reflexes (e.g., chick amnion, Evans and Schild, '56) and many smooth muscles show increased excitability when they are extended (e.g., taenia coli of guinea pig, Bulbring, '54).

Is conduction in visceral smooth muscle of lower vertebrates and invertebrates independent of nerves? What is the relation between mechanical pull and conduction in muscles which are stimulated by stretch?

MATERIALS AND METHODS

External recording was by low-resistance wicks and silver-silver chloride electrodes connected to a high-gain amplifier. Mechanical activity was detected by an RCA transducer or by a Grass strain gauge. Most of the external recording was displayed on a Grass polygraph fed by a chopper amplifier. Mechanical stretches and releases were applied by a magnetic device which could deliver a pull of a predetermined number of millimeters at a rate of 1 mm per msec. Intracellular recording was with microelectrodes by methods given in the preceding paper for the proboscis retractor of *Golfingia*, (Prosser and Sperelakis, '59).

Two other muscles were used besides the proboscis retractor of *Golfingia*. The intestine of *Golfingia* spirals around a thin muscle, the spindle (fusiform) muscle or intestinal retractor (Andrews, 1890). The ascending loop of the intestine forms a helix around this muscle and is attached to it, while the descending loop follows the ascending one in its coils, but it not attached to the muscle. The spindle muscle originates in the body wall near the anus and terminates at the region of the intestine where the ascending loop begins.

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[1] Gratitude is expressed to Mr. Russell Close who constructed and helped us employ the instrument used for quick stretch and release in many of the experiments.

[2] Supported in part by NIH contract with MBL.

[3] Since an extensive experimental literature concerns the New England sipunculid, now known as *Golfingia*, formerly *Phascolosoma* (Fisher, '52), the name phascolosoma is here adopted as a common name.

No connection with the cerebral ganglion or the ventral nerve cord could be found. The muscle is so small that most electrical recording was done under mineral oil.

The mesentery of the smooth dogfish *Mustelus canis* contains a sheet of smooth muscle which pulls the spiral valve of the upper intestine toward the dorsal body wall (Cannon, '08). Rhythmic contractions have been noted in response to stretch; adrenaline, acetylcholine and pituitrin elicit contractions (McKay-Sawyer, '33). Strips of mesenteric muscle were isolated in dogfish saline containing urea (Babkin et al., '33).

RESULTS

The spindle muscle (intestinal retractor) of Golfingia

When the spindle (fusiform) muscle was stimulated electrically, external recording showed complex action potentials, mainly fast components. Sometimes a single shock initiated repetitive bursts of potentials, each burst associated with separate contractions. On repetitive stimulation at a low frequency (0.1/second) the muscle tended to follow without either the fatigue or facilitation seen in the proboscis retractor. The velocity of conduction, as measured by dual channel recordings from two pairs of electrodes, was slow — average 0.73 cm/second (fig. 1a).

The excitability of the spindle muscle varied with stretch; when allowed to hang slack it was not readily excited; when under tension it was more readily stimulated either electrically or by touching with a glass rod (fig. 2 a-c). The electrical response was more persistent (repetitive) when taut than when relaxed. Also when taut, the muscle often showed rhythmic

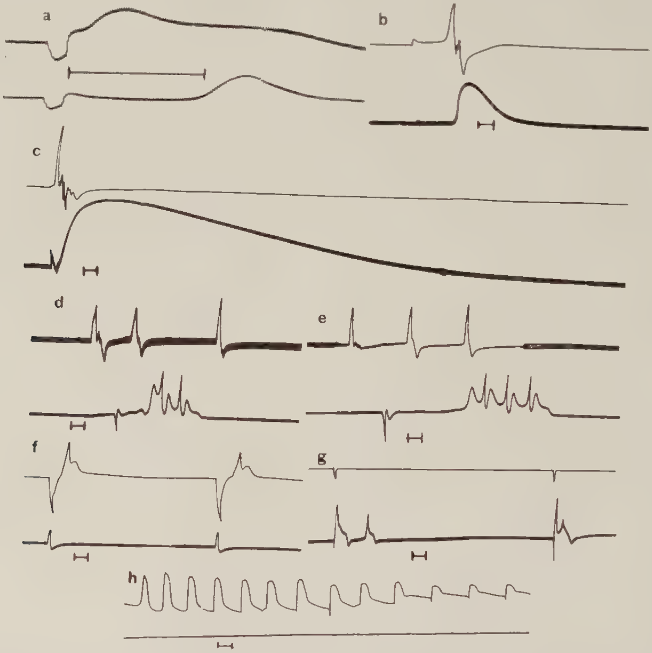

Fig. 1 Responses of spindle muscle. Time marks one second. a, action potentials in response to shock recorded simultaneously from two regions 15 mm apart for velocity measurement; b, c, electrical response above, mechanical below, responses to stretch, b before and c after 10 minutes soaking in tetracaine 10^{-4}; d; e, electrical responses recorded from two muscle regions connected by taut oil-soaked thread, upper record from segment receiving electrical stimulation, lower record from muscle on other side of thread; f, g, h, muscle coiled around glass rod to prevent movement, in f, upper record from portion of muscle on side of rod receiving stimulation, lower record from region on other side of rod, only stimulus artifact conducted, in g stimulus applied on opposite side from f, in h, spontaneous activity on one side of rod (upper record) not transmitted to portion of muscle on opposite side (lower record).

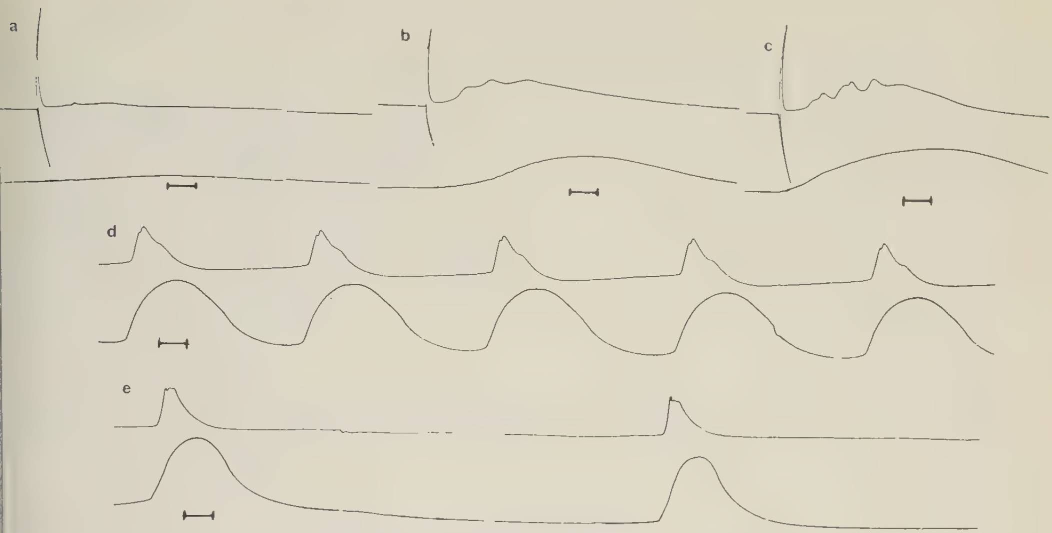

Fig. 2 Spindle muscle. Time records one second. a, b, c, action potentials above, contractions below in response to shocks, stimulus artifact initial downward deflection, response upward; a to c, increasing tension applied to muscle; d, e, spontaneous rhythms, electrical above, mechanical below, tension on muscle higher in d than in e.

spontaneous activity at one per second to one per three seconds (fig. 2 d-e).

When a 2- to 4-cm length of the spindle muscle was quickly stretched by 1 to 4 mm, it responded by an active contraction after a brief latency. The latency lengthened and the amplitude of contraction decreased as the stretch was diminished and a threshold amount of stretch was necessary to stimulate (fig. 3 h, i, j, k). Often a stretch well above threshold initiated rhythmic contractions (fig. 3a). Action potentials preceded and accompanied the mechanical responses to quick stretch and showed a velocity of a little less than 1 cm per second (fig. 3 b, c). If released during contraction, tension was redeveloped to nearly the level corresponding to the phase of contraction (fig. 3 d, e, f, g).

The very slow velocity of conduction, the sensitivity to tension and the active response to stretch suggest that propagation in the spindle muscle is by mechanical pull from fiber to fiber. To block mechanically, a muscle was wrapped around a 1-mm glass rod. Under conditions where no rotation of the muscle on the rod occurred, a contraction wave was conducted up to the block and no electrical or mechanical response was seen beyond it (fig. 1 f, g, h). In other experiments, an oil-soaked thread was tied between two cut portions of a muscle. When the muscle and thread were taut so that pull could be transmitted through the thread and the muscle at one end was stimulated, electrical responses were obtained from muscle on both sides of the thread (fig. 1 d, e). If a short thread was tied between two points on a muscle so that a long mid-region hung loosely below, a response was obtained from the tensed portion beyond the thread but no response from the loose portion below the thread even though this region was nearer the stimulating electrodes. It is concluded that propagation in the spindle muscle is by mechanical pull from one region to the next.

The effect of stretch on the spindle muscle might be on muscle fibers or it might involve nerves or possibly an intrinsic reflex. Worms were injected with methylene blue, which stains nerves in the proboscis retractor very well. No connection of the muscle with the central nervous system was noted. A few nerve fibers were seen in the mesentery between the muscle and the intestine. In the center of the spindle muscle a core was stained with methylene blue. Histological sections showed this core to consist of coarse fibers which did not take a silver stain and which stained

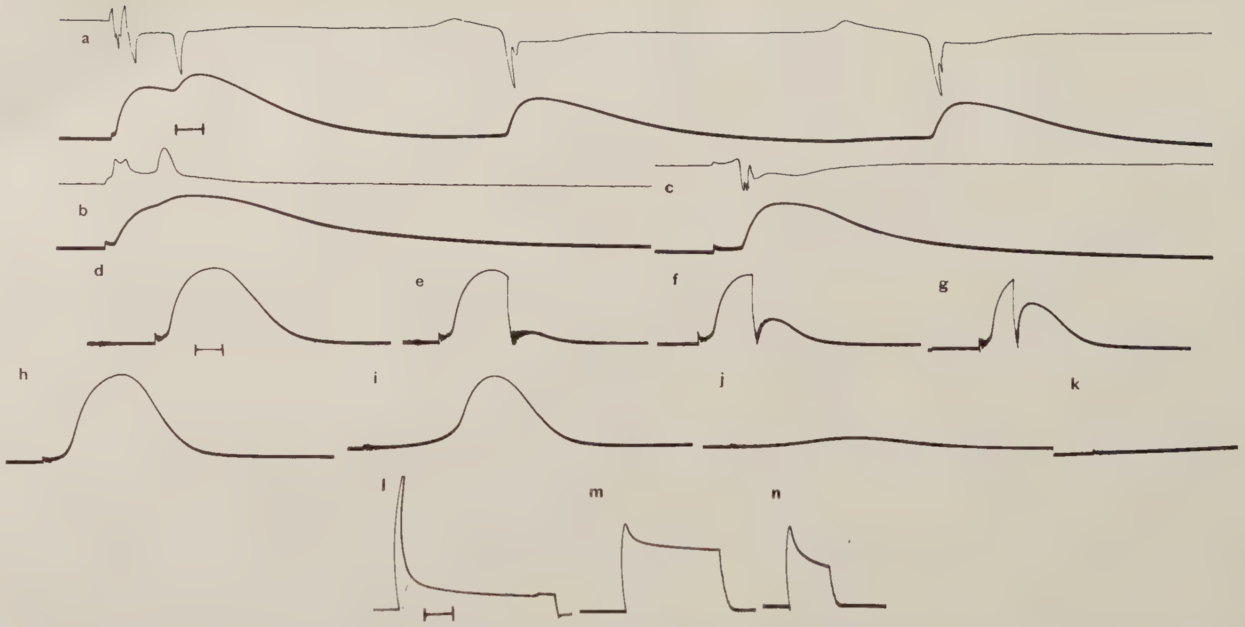

Fig. 3 a-k, Spindle muscle, 1-n, proboscis retractor. a, b, c, responses initiated by stretch to 1.8 cm long muscle, electrical response above mechanical below, stretch in a, was 4 mm, response rhythmic, b stretch reduced to 2.5 mm, in c 1.5 mm; d, e, f, g, mechanical responses to quick stretch, d, complete contraction, e, f, g quick release earlier in contraction e to g; h, i, j, k, responses of 2.3 cm long muscle to stretch in h 3.5 mm, i 2.25 mm, j 1.75 mm, k 1.5 mm; 1, m, n, effects of quick stretch and release on proboscis retractor, l 1.3 mm stretch and release, m 5.5 mm stretch and release of a 5.8 cm long muscle, n 5.5 mm stretch and release of 5.3 cm muscle, time line one second.

like the outer connective tissue sheath with Mallory's triple stain. Also, the core failed to stain with thionin or sudan black. It is concluded that the core is not nerve fibers but is probably connective tissue.

The proboscis retractor shows nerve degeneration and loss of conduction after several days in the refrigerator. When spindle muscles were similarly treated for 5 to 6 days they still responded to shock and stretch and showed relatively normal conduction. In some of these the central core failed to stain, in others it did stain with methylene blue.

The nerve-blocking drug tetracaine stops conduction in the proboscis retractor; a concentration of 10^{-4}, applied locally, blocked in less than 15 minutes. When tetracaine at the same concentration was applied to the fusiform spindle muscle, there was no block at 30 minutes. When soaked in tetracaine the threshold was raised and responses could be stopped irreversibly at a concentration of 10^{-4} and reversibly at 10^{-5}. After 10 to 15 minutes in 10^{-4} tetracaine the contraction time was lengthened from 1–1.5 to 4–6 seconds and the total duration of contraction from 8–10 seconds to 40–50 seconds (fig. 1 b, c). Tetracaine can raise threshold and prolong contraction but local applications which block nervous conduction in the proboscis retractor do not block the spindle muscle. D-tubocurarine blocks the response of the proboscis retractor at the point of application (Prosser and Melton, '54). At the concentration of 10^{-4} it had no effect on the spindle muscle (30 minutes). Acetylcholine at 10^{-5} stimulated the muscle to contract.

Important evidence for nerve conduction in the proboscis retractor was observation of nerve impulses at the same velocity but well ahead of the muscle responses (Prosser and Melton, '54). Action potentials were recorded from the spindle muscle with high gain amplification and various sweep speeds of the oscilloscope. The responses were usually smooth, sometimes complex, much as when recorded with the ink-writer (fig. 4 a, b, d, e, f). There was no evidence of any nerve impulses ahead of the muscle response. If the central core were a nerve, some evidence of fast action potentials

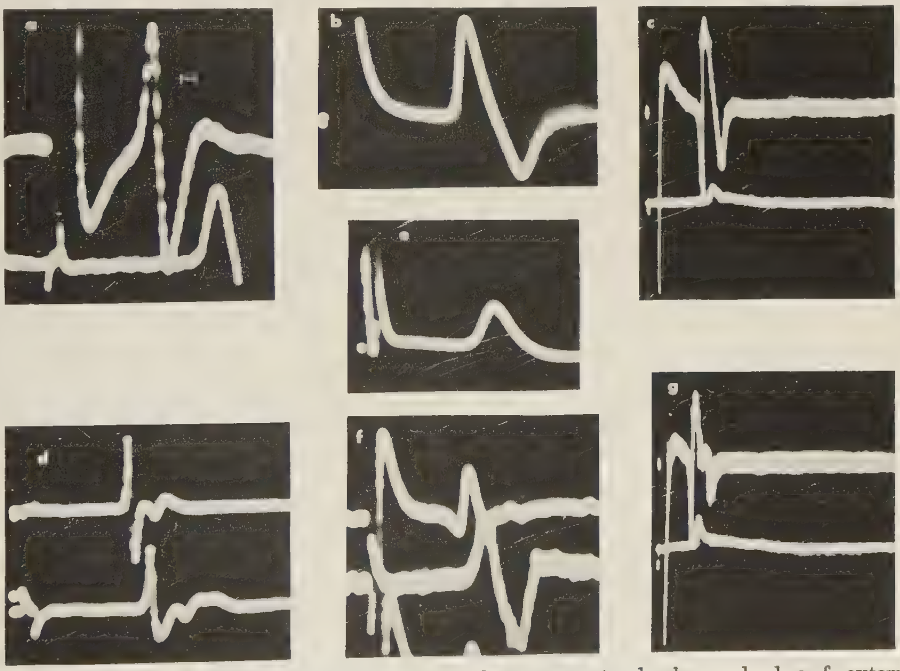

Fig. 4 Spindle muscle. Oscilloscope records of responses to shocks. a, b, d, e, f, external recording, a, d, f simultaneous recording from two regions. c, g, externally recorded response upper beam, intracellular response lower beam.

from it should have been found. The evidence from the histology, the effects of drugs, and the high-speed recording indicates that the response to stretch which mediates conduction in the spindle muscle resides in the muscle fibers and is not a nervous reflex.

Many attempts were made to record with microelectrodes from this muscle. Macerated muscles gave fiber dimensions of an average maximum width of 5.7–6 μ and length of 800–900 μ. These fibers are a little narrower and much longer than those of the proboscis retractor. To penetrate them with microelectrodes proved very difficult. High resistance electrodes of various tip shapes were tried repeatedly with both rigid and floating manipulation. The largest "resting" potentials recorded were 10 mv and few reliable action potentials were obtained. These were spikes without overshoot (fig. 4 c, g). It is probable that the fiber membranes do not permit an adequate seal around a microelectrode tip.

The proboscis retractor of Golfingia

Evidence has been presented previously that propagation in the proboscis retractor is by nerves (Prosser and Melton, '54) and that many of the muscle fibers are dually innervated (Prosser and Sperelakis, '59).

When a proboscis retractor was stretched, there was initial elastic tension which declined rapidly to a value corresponding to the new length. There was no active response to the stretch but rather the muscle reacted to stretch much the same as a vertebrate striated muscle (fig. 3 l, m, n). Conducted impulses pass a region of mechanical block; hence pull is not necessary for propagation.

The mesenteric muscle or spiral valve retractor of the dogfish

This muscle consists of typical vertebrate smooth muscle fibers 2 to 3 μ diameter and 0.1 to 0.18 mm long as measured from macerated tissue. The fibers are grouped in fasciculi, each about 0.1 to 0.2 mm wide. Strips about a half to two millimeters wide and 2 to 4 cm long were mounted in dogfish saline for stretch stimulation and for microelectrode recording, or in paraffin oil or moist air for external recording.

When stimulated with a single or with several suprathreshold shocks an initial fast spike or group of spikes appear on an external electrical record, then a train of smaller spikes as contraction starts (fig. 5 a, c, 7 a, b). Contraction may be maintained for from one half minute to several minutes with very little electrical activity, but as relaxation begins, increased electrical activity is noted (fig. 5 b, d, e, f). Shocks applied during the maintained contraction elicit no apparent response. Much variability occurred, some preparations showed more rapid relaxation than others and some showed continued action potentials during contraction and not during relaxation. Some preparations showed relatively brief slow potentials and these could be repeated at intervals greater than 5 seconds. Long recovery periods (about 10 minutes) were necessary between successive large contractions showing multiple action potentials.

With two pairs of recording electrodes, velocities were measured at an average of 1.1 cm/second at 23°C (fig. 6 e). This is of the same order as the velocity of mammalian smooth muscle (3 to 5 cm/second at 37°C) but much slower than the nerve transmission of the phascolosoma proboscis retractor and faster than the stretch conduction of the spindle muscle. Responses of the dogfish mesenteric muscle were better under slight tension, but were obtained irrespective of whether the muscle was taut or loose.

When subjected to a quick stretch, a response was initiated which resembled that to a shock. A burst of spikes was recorded and then an active contraction which lasted for more than 1.5 minutes and with little maintained electrical activity (fig. 6 a, b). Sometimes repetitive action potentials appeared during relaxation (fig. 6 a, b). Responses were graded according to the stretch. Contractions were steady and only rarely repetitive as in the spindle muscle. If during a contraction, tension was suddenly released, there was redevelopment of tension to near the pre-release level (fig. 6 b, c). If release occurred near the beginning of relaxation, there was little or no redevelop-

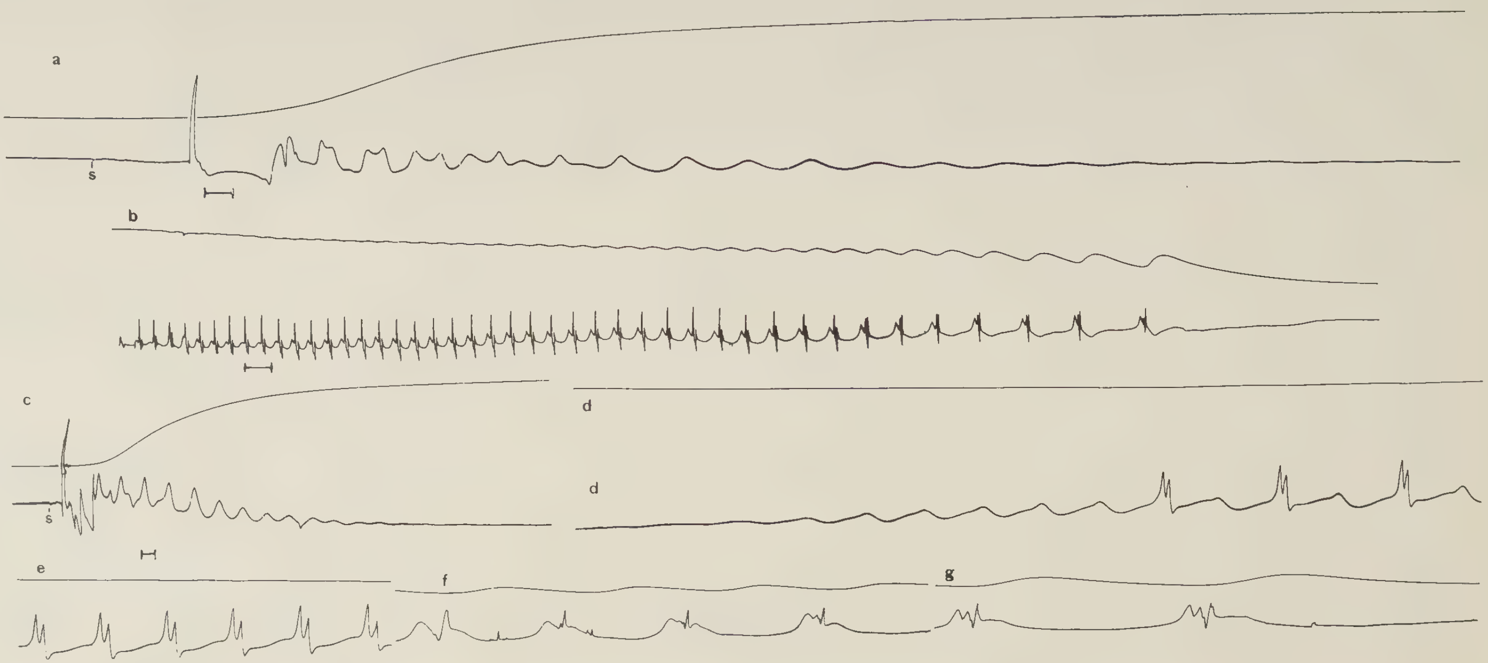

Fig. 5 Dogfish mesenteric muscle; time lines one second, a, b, continuous response to shock at s, contraction above, action potentials below, between a and b 120 seconds of record omitted and paper speed reduced. c, d, e, f, g, response to shock at s, mechanical response above, electrical below, between c and d 115 seconds omitted, between e and f 125 seconds omitted, between f and g 35 seconds omitted, paper speed uniform throughout.

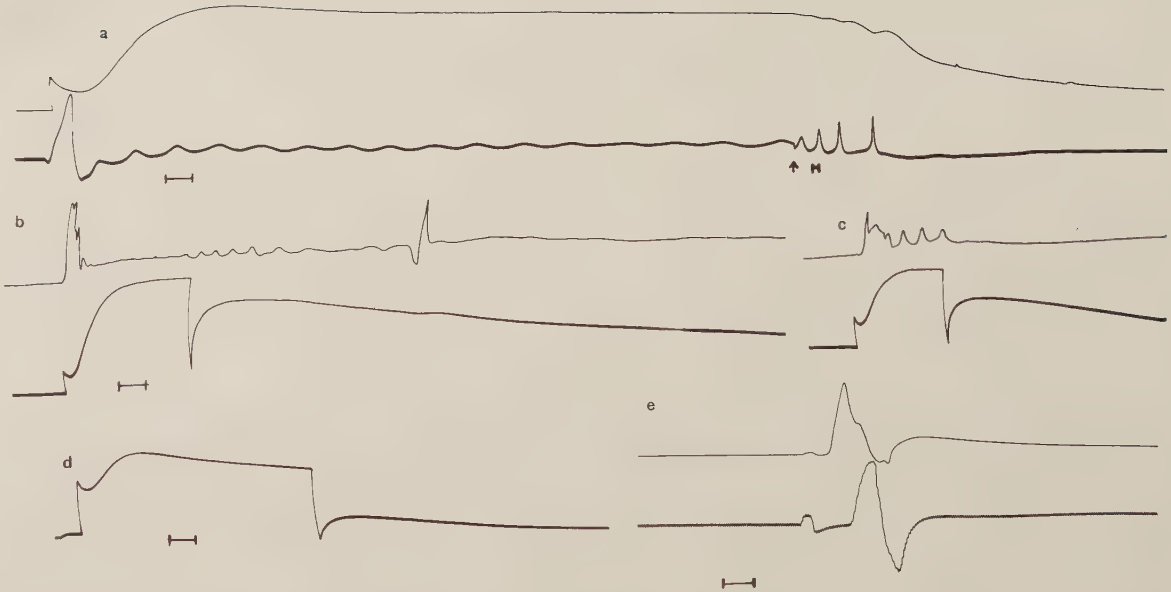

Fig. 6 Dogfish mesenteric muscle, responses to quick stretch; time lines one second. a, response of 2.8 cm strip of muscle to 4 mm stretch indicated by first upward deflection, contraction above, action potentials below, paper speed slowed at arrow near end of record; b, response to stretch, electrical response above, mechanical below, release after maximum tension attained was followed by electrical activity and redevelopment of tension; c, contraction (below) and action potentials (above) in response to stretch, release after peak tension; d, contraction in response to stretch followed by release after relaxation began; e, action potentials recorded from two regions 14 mm apart, electrical stimulation.

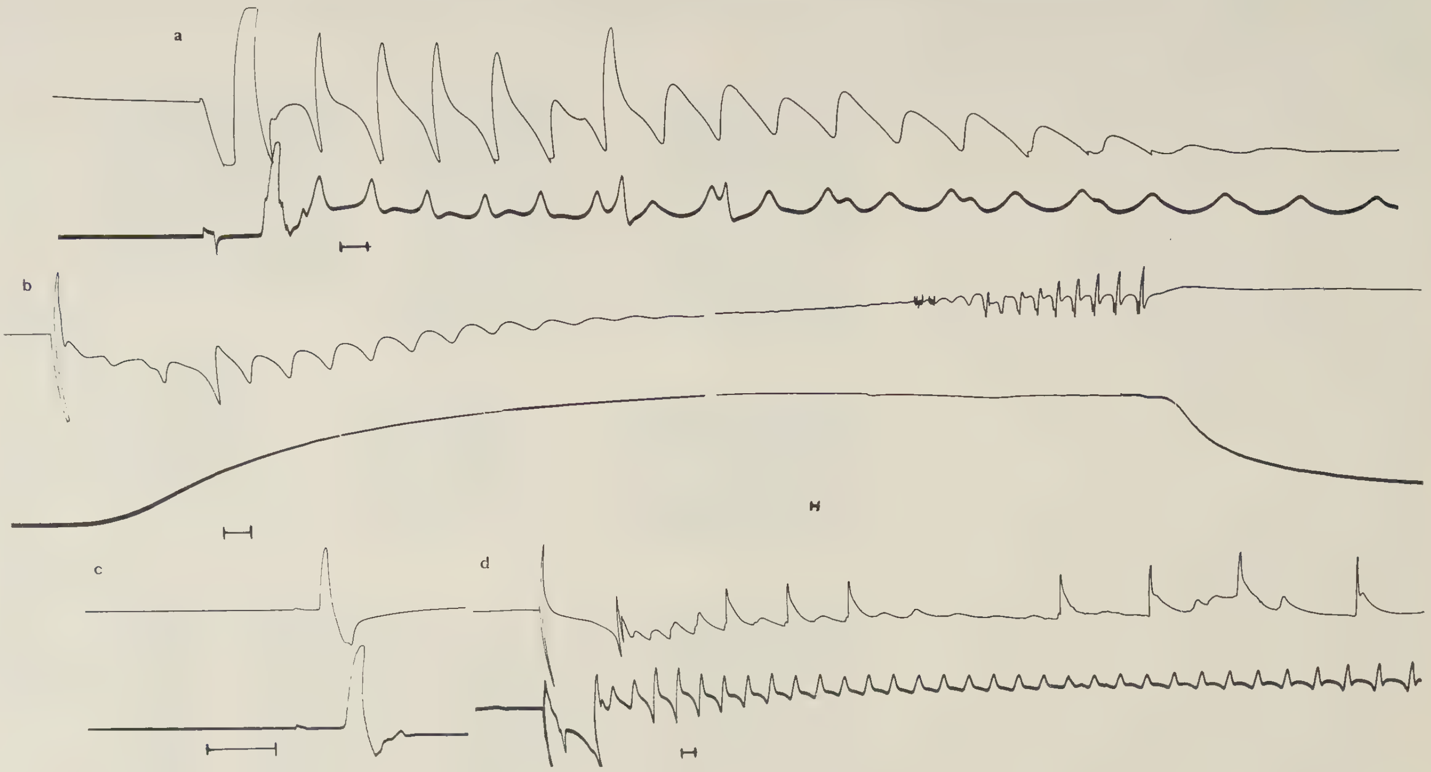

Fig. 7 Repetitive responses of dogfish mesenteric muscle. Time marks one second. a, action potentials in response to shock, records from two regions 15 mm apart; b, contraction below and electrical response above to single shock, at break in record three seconds removed and paper speed reduced; c, electrical responses recorded from muscle wrapped around rod to prevent movement, stimulus and upper response at left, lower response at right of rod; d, rhythmic electrical responses recorded on two sides of immobilizing rod to stimulus applied on left side (lower record).

ment of tension (fig. 6 d). Apparently the active state persists until shortly before relaxation.

When a strip was immobilized in the middle by winding around a small glass rod, good propagation at the appropriate velocity was observed from one side to the other (fig. 7 c, d). Propagation does not, therefore, require mechanical pull, although stretch can stimulate the muscle.

After two days in saline in the refrigerator, good conducted responses were still obtained. D-tubocurarine at 10^{-4} did not block conduction, nor did tetracaine at 10^{-4} applied for some 20 minutes. Gamma-amino-butyric acid at 10^{-4} to 10^{-5} prolonged contractions, enhanced the electrical responses and tended to make them repetitive. Stimulation by acetylcholine and by adrenaline has been reported previously (McKay-Sawyer, '33).

Staining with methylene blue revealed scattered thin nerve fibers, presumably autonomic ones. Ganglion cells were not seen. Action potentials were recorded by external electrodes at high gain and fast oscilloscope sweep. Responses were smooth or multiple and showed no evidence of nerve impulses ahead of the muscle response (fig. 8, c, h, i). In this respect they resemble responses of mammalian deganglionated intestinal muscle rather than the intact ureter (Prosser, Smith and Melton, '55; Prosser and Sperelakis, '56).

From the velocity, the insensitivity to tetracaine, the scarcity of nerves, the absence of nerve spikes ahead of muscle potentials, and the propagation through a mechanical block, it is concluded that propagation in the dogfish mesenteric muscle is independent of nerves and of mechan-

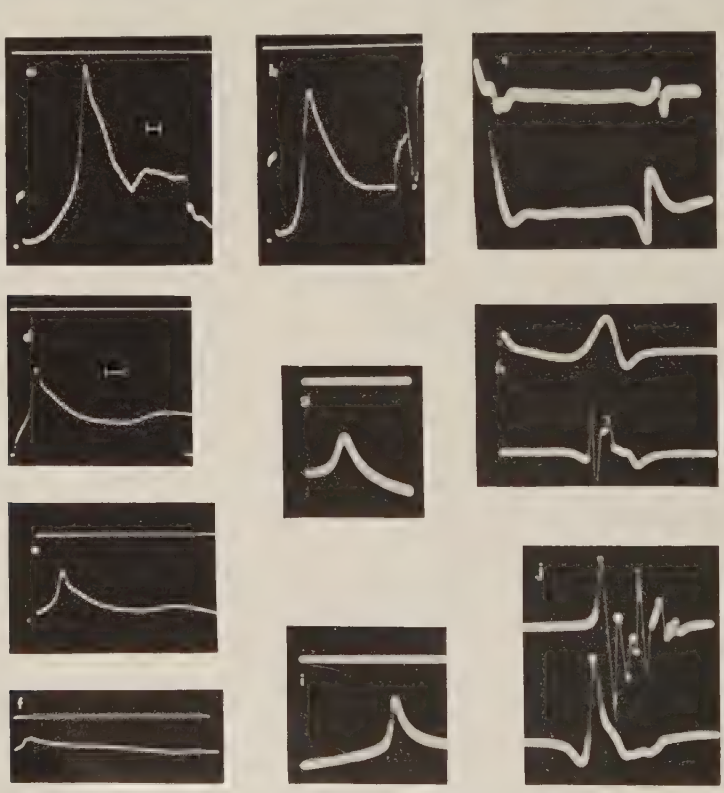

Fig. 8 Oscilloscope records from dogfish mesenteric muscle, time record (a, d) 0.1 second; a, b, intracellular responses to shock, resting potential 48 mv in each; c, h, i, externally recorded responses at two points to shock stimuli; d, e, f, series of intracellular responses to shocks as recorded resting potential declined, initial resting potential (d) 39 mv; g, i, intracellularly recorded potentials during a maintained tension, resting potential 32 mv.

ical pull but is, like that of mammalian smooth muscle, from muscle fiber to fiber.

Microelectrodes were relatively easily inserted into the dogfish smooth muscle. Despite the small fiber diameters, many penetrations yielded high resting potentials. The average of 24 measurements was 70 mv with a range of 32 to 75 mv. This is higher than is usually recorded from mammalian smooth muscle. The difference in ease of penetration and length of holding of a resting potential of the dogfish muscle as compared with the *Golfingia* muscles with the same microelectrodes was striking and indicates differences in the mechanical properties of the membranes.

Some action potentials were recorded intracellularly during maintained tension (fig. 8 g, i), and in response to shocks (fig. 8 a, b, d). These were simple spikes 0.5 second in duration. They did not overshoot zero potential, but decreased as the observed resting potential decreased, presumably during withdrawal (fig. 8, d, e, f). They resembled the intracellular spikes of many mammalian smooth muscles.

DISCUSSION

The three muscles considered above have fibers of similar size and shape, yet they represent three different methods of propagation of activity. In the phascolosoma proboscis retractor, conduction is entirely by nerves and the electrical responses of the muscle fibers appear to be junction-type potentials. Nerve conduction has been indicated in non-striated muscles of holothurians, annelids, molluscs and coelenterates (Prosser, '59 for references). It is probable that all somatic non-striated muscles of invertebrates are activated by nerves. This is one important reason for avoiding the term smooth muscles for these short-fibered invertebrate muscles.

In the spindle muscle or intestinal retractor of phascolosoma, unlike the proboscis retractor, conduction is independent of nerves but is by mechanical pull from one region to the next. This muscle is excited to graded responses by quick stretches and is unusually sensitive to mechanical stimulation. The proboscis retractor, in contrast, responds passively to stretch much like striated muscle. The active response of the spindle muscle is direct rather than reflex. Why the fiber membranes of this muscle are so sensitive to mechanical deformation while those of the proboscis retractor are not, is an unsolved problem.

The dogfish mesenteric muscle or spiral valve retractor has properties like those of visceral smooth muscles of mammals. The velocity, absence of nerve impulses, insensitivity to nerve blocking drugs agree in indicating conduction from fiber to fiber through the muscle. Conduction passes a region of mechanical block as in cat intestinal muscle (Sperelakis and Prosser, '59). The dogfish muscle, however, does respond actively to mechanical stretch as does the chick amnion. The nature of the conduction process in smooth muscle is not fully known, but there appears to be electrical continuity between fibers. In no invertebrate non-striated muscle, somatic or visceral, has propagation of the vertebrate smooth muscle type been found.

The resting potentials of the dogfish smooth muscle are larger than those recorded for mammalian smooth muscle (50–60 mv in guinea pig taenia coli, Holman, '58). They contrast with the low values for the phascolosoma muscles. The differences may be due partly to true differences in resting potentials and partly to different mechanical properties of the cell surfaces making for variable shunting around the electrodes.

SUMMARY

1. The non-striated spindle muscle (intestinal retractor) of *Golfingia* (= *Phascolosoma*) gives single or repetitive responses to single shocks. Excitability is higher and tendency to rhythmic response is greater when the muscle is taut than when slack.

2. Quick stretch of the spindle muscle elicits fast action potentials and an active contraction, the magnitudes of which vary with amount of stretch and initial tension. Release of a contracted muscle is followed by redevelopment of tension.

3. Propagation is slow (0.73 cm/second) and does not go beyond a region of mechanical block; there can be propaga-

tion from one portion of muscle to another even when separated by a taut thread.

4. Tetracaine and D-tubocurarine, also storage in cold for 6 days fail to abolish propagated responses. No nerves were found in the muscles.

5. Penetration of the muscle fibers with microelectrodes proved very difficult, only small "resting potentials" were recorded and action potentials were graded.

6. The proboscis retractor of *Golfingia* shows passive elastic tension when stretched but no active response.

7. The mesenteric muscle (spiral valve retractor) of a dogfish may remain contracted for as long as several minutes in response to a single stimulus. Repetitive action potentials occur during contraction, there may be little or no electrical activity during maintenance of tension and renewed activity during relaxation.

8. The mesenteric muscle responds with an active contraction to quick stretch and redevelops tension after quick release during contraction.

9. Propagation is at 1.1 cm/second and passes a region of mechanical immobilization. Mechanical pull is not necessary for propagation. Nerve inactivating drugs (tetracaine, D-tubocurarine) failed to block propagation.

10. Penetration of the mesenteric muscle fibers was easier than with either of the *Golfingia* muscles; resting potentials averaged 70 mv. Intracellular action potentials were simple deflections without overshoot.

11. It is concluded that in these three non-striated muscles, propagation in the phascolosoma proboscis retractor is by nerves, in the spindle muscle propagation is by mechanical pull of one fiber on the next, and in the dogfish mesenteric muscle conduction is from muscle fiber to fiber much as in mammalian smooth muscle.

LITERATURE CITED

Andrews, E. A. 1890 Notes on the anatomy of *Sipunculus gouldii*, Pourtales. Studies from Biol. Lab. Johns Hopkins Univ., *4:* 389–430.

Babkin, B. P., D. J. Bowie and J. V. V. Nicholls 1933 Structure and reactions to stimuli of arteries (and conus) in the elasmobranch genus, *Raja*. Contr. Canad. Biol. and Fish., N.S., *8:* 207–225.

Bozler, E. 1948 Conduction, automaticity and tonus of visceral muscles. Experientia, *4:* 213–218.

Bulbring, E. 1954 Membrane potentials of smooth muscle fibers of the taenia coli of the guinea pig. J. Physiol., *125:* 302–315.

Cannon, W. B. 1908 The movements of the alimentary canal in the dogfish. Amer. Nat., *42:* 326–327.

Evans, D. H. L., and H. O. Schild 1956 Reaction of chick amnion to stretch and electrical stimulation. J. Physiol., *132:* 31P.

Fisher, W. K. 1952 Sipunculid worms of California and Baja California. Proc. U. S. Nat. Mus., *102:* 371–450.

Holman, M. E. 1958 Membrane potentials recorded with high-resistance micro-electrodes; and the effects of changes in ionic environment on the electrical and mechanical activity of the smooth muscle of the taenia coli of the guinea pig. J. Physiol., *14:* 464–488.

McKay-Sawyer, M. E. 1933 A mechanism controlling movement of the spiral intestine in elasmobranchs. Contr. Canad. Biol., *7:* 479–494.

Prosser, C. L. 1959 Comparative physiology of neuromuscular transmission with particular emphasis on non-striated muscles. In: Muscle, ed. G. Bourne, Academic Press, New York, 1960.

Prosser, C. L., and C. E. Melton 1954 Nervous conduction in smooth muscle of Phascolosoma proboscis retractors. J. Cell. and Comp. Physiol., *44:* 255–276.

Prosser, C. L., C. E. Smith and C. E. Melton 1955 Conduction of action potentials in the ureter of the rat. Am. J. Physiol., *181:* 651–660.

Prosser, C. L., and N. Sperelakis 1956 Transmission in ganglion-free circular muscle from the cat intestine. Am. J. Physiol., *187:* 536–545.

——— 1959 Electrical evidence for dual innervation of muscle fibers in the sipunculid Golfingia (=Phascolosoma). J. Cell. and Comp. Physiol., *54:* 129–134.

Sperelakis, N., and C. L. Prosser 1959 Mechanical and electrical activity in intestinal smooth muscle. Am. J. Physiol., *196:* 850–856.

The Influence of Temperature on the Kinetics of Ryanodine Contracture[1]

WILFORD L. HASLETT AND DONALD J. JENDEN
*Department of Pharmacology, UCLA Medical Center,
Los Angeles 24, California*

The contracture which ryanodine produces in frog striated muscle has been shown to be delayed quite markedly by cooling the muscle to 4°C; the normal time course is resumed when it is brought back o the environmental temperature of 23°C (Edwards and Flinker, '50). According to Ramsey et al., ('50), cooling to 4°C may prevent shortening completely for periods exceeding 24 hours, in the presence of a concentration of ryanodine (10^{-5} gm/ml) which causes maximum contracture in about 30 minutes at 23°C. In a more detailed study of the influence of temperature on the time required for onset of the ryanodine contracture, this author reached the conclusion that the Q_{10} exceeds 8.36 in frog muscle (Ramsey, '56).

The work presently reported is a quantitative study of the relation between ryanodine concentration, temperature, time and muscle length in the isolated rat diaphragm preparation. Its primary object was to obtain some clue as to the possible mechanism of action of ryanodine by defining more accurately the factors controlling the kinetics of its effect and the relations between them.

METHODS

Wistar rats weighing 130–180 gm were used. Two diaphragm preparations were obtained from each animal, one from each side; each was dissected in the conventional way and arranged as quickly as possible on a plastic support in Krebs bicarbonate solution aerated with 95% oxygen and 5% carbon dioxide. An equilibration period of approximately 30 minutes was allowed before the ryanodine was added. The contracture was recorded kymographically with isotonic levers, using a tension of 1 gm.

Temperature regulation was achieved by the use of water-jacketed baths. Water was pumped in series through these from a reservoir containing a toluene thermoregulator, heater and cooling coil. There was no detectable difference between the temperatures of the two tissue baths.

In the experiments requiring electrical stimulation, this was achieved by means of longitudinally placed platinum mass electrodes and a Grass square-wave stimulator.

Experimental design

Six series of experiments were conducted at different temperatures ranging from 16.1°C to 40°C. Seven different ryanodine concentrations were studied in each series; these were geometrically spaced. In view of the extreme temperature effects encountered, and the necessity of maintaining the time course of the contracture within reasonable bounds, the ryanodine concentrations were not the same at each temperature, but the spacing remained the same (ratio of approximately 1.6 between concentrations). Four replicates were obtained for each pair of values of concentration and temperature; two from left diaphragm preparations and two from right preparations. The sequence in which the different concentrations were tested was chosen so that the temporal order of the experiments was orthogonal to the order of increasing concentration, and the two sides of the diaphragm were always exposed to different concentrations.

A second set of experiments was also carried out in which direct electrical stimulation was applied to the muscle at a

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rate of 6 per minute, to determine the influence of intermittent activity on the kinetics of contracture. Only three ryanodine concentrations were used at each temperature in this series, corresponding to the highest, central and lowest concentrations in the unstimulated series. Other details of the experimental design were the same.

RESULTS

The time course of isotonic ryanodine contracture in the rat diaphragm has been previously described (Blum et al., '57). A latent period, during which very little shortening occurs, precedes the contracture, and the shortening persists indefinitely once it is complete, although an extremely slow and presumably passive stretching may occur in the course of several hours. The relation between time and fractional shortening is approximately uniform over all the concentrations and temperatures studied, if the time scale is adjusted to make the half times of shortening coincide. The entire curve may therefore be adequately specified by this rate parameter. Figure 1 summarizes the data obtained on unstimulated muscle; a logarithmic plot was employed semi-empirically so that the entire complex of data may be seen simultaneously.

The data are adequately represented by a series of straight lines with a mean slope of 0.558. The lines are not quite parallel but have a slightly higher slope at the higher temperatures. This change in slope is small (0.098/°C) but statistically significant (table 1, component B) and is apparent in the smaller series of experiments on stimulated muscle as well as resting muscle. Its meaning will be considered further in the discussion.

Table 1 also shows that the size of the rat from which the preparation was taken exerts a significant effect on the rate of contracture (component C). This effect was highly significant only in the series of experiments at 40°C (table 2). Here it was surprisingly pronounced, a 10-gm increase in weight corresponding to a 20% decrease in the expected half time of contracture. The series at 20.6°C was significant at a lower level (1%), but this result seems anomalous and may represent random variation.

A similar, but less extensive, set of data was obtained during direct stimulation of the muscle at a rate of 6 per minute. These are summarized in figure 2. The mean slope in this series was 0.512 and there was again a tendency for the slope to be higher at the higher temperatures. Vertical cross sections of figures 1 and 2

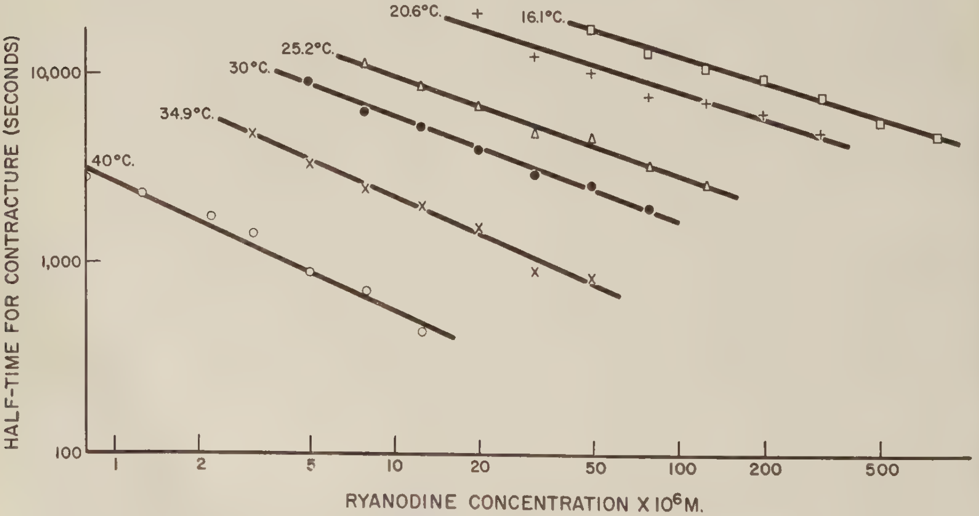

Fig. 1 Series of lines showing relation between half time of contracture, ryanodine concentration, and temperature in unstimulated rat diaphragm. Each point represents the mean of 4 experiments.

TABLE 1

Analysis of variance for effect of ryanodine concentration and temperature on $\log_{10}$ *(half time) of contracture of unstimulated rat diaphragm*

	Source	SSq	D.F.	MSq	F	P
	Within temperatures					
A	Regression on log (dose)	8.35812	1	8.35812	1092.6	$<< 0.001$
B	Deviation from parallelism					
B1	Regression of slope on temperature	0.17008	1	0.17008	22.233	< 0.001
B2	Residual	0.01195	4	0.00299	0.391	> 0.2
C	Regression on weight of rat	0.08424	1	0.08424	11.012	<0.01
C1	Deviation from parallelism	0.56455	5	0.11291	14.759	< 0.001
D	Difference between left and right diaphragms	0.00224	1	0.00224	0.314	> 0.2
E	Residual	1.13947	149	0.00765		
	Between temperatures					
F	Regression on temperature	18.68176	1	18.68176	2442.1	$<< 0.001$
G	Residual	1.22606	4	0.30652	40.068	< 0.001
	Total	30.23847	167			

TABLE 2

Influence of rat weight on rate of ryanodine contracture. Standard errors were computed using component E, table 1 as estimate of random error

Temperature	Partial regression coefficient $\log_{10}$ (half time) on wt. of rat
	gm × 10^3 ± *S.E.*
40.0	−8.20 ± 1.09[2]
34.9	−2.97 ± 1.25
30.0	−0.95 ± 0.93
25.2	−1.43 ± 1.17
20.6	+3.44 ± 0.84[1]
16.1	−1.80 ± 0.93

[1] $P < 0.01$.

[2] $P \ll 0.001$.

allow an Arrhenius plot of the $\log_{10}$ (half time) against the reciprocal of the absolute temperature at constant ryanodine concentration (fig. 3). These plots are neither linear nor parallel, but indicate that stimulation accelerates the ryanodine contracture more markedly at lower temperatures than at 40°C.

It should be pointed out that the slopes of these Arrhenius plots are to some extent dependent on the position on the abscissa of figures 1 and 2 at which the cross sections are taken, since the lines on these figures are not quite parallel. The change is small, however, and the shape of the Arrhenius plot is similar wherever the cross section is taken.

DISCUSSION

The results presented confirm the previously reported large temperature coefficient of ryanodine induced contracture in skeletal muscle (Edwards and Flinker, '50; Ramsey et al., '50; Ramsey, '56). Since an Arrhenius plot of the data does not give straight lines (fig. 3), it is likely that no one process is rate-limiting over the temperature range studied. The two lines in figure 3 are approximately parallel at the upper end of the temperature range, the slope corresponding to an apparent activation energy of about 48,000 cals mol^{-1}. At lower temperatures the slope of both lines falls off, and corresponds to apparent activation energies of 20,000 and 8,000 cals mol^{-1} for the unstimulated and stimulated series respectively.

It is of considerable interest that the slopes decrease with decreasing temperature; in a catenary system of several processes, the reverse is almost universally true (Johnson, Eyring and Polissar, '54). Although it is possible to construct a model series of several processes, the net rate of which would have a temperature coefficient which increased with temperature, this situation may arise more simply when there are two alternative causal pathways leading to the same effect (Booij and Wolvekamp, '44; Stearn, '49). In this case, the rate of the faster process will have the

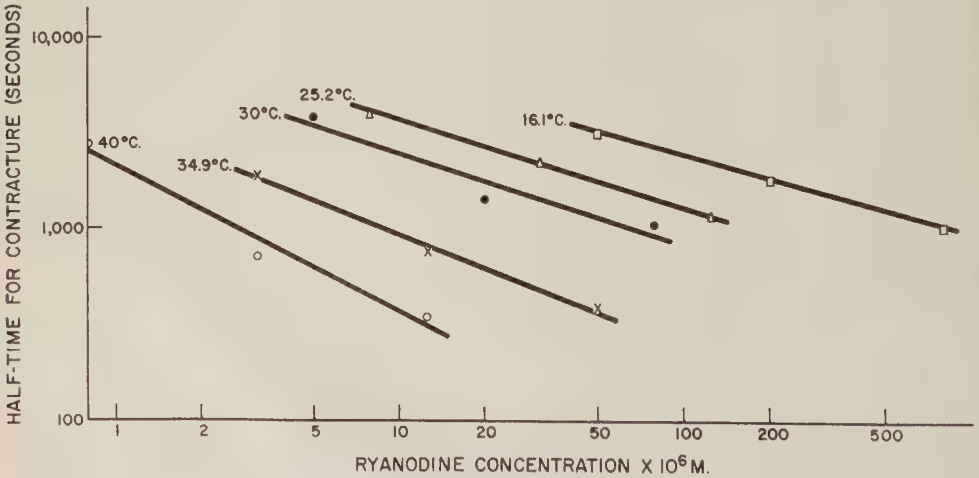

Fig. 2 Series of lines showing relation between half time of contracture, ryanodine concentration, and temperature in rat diaphragm stimulated directly every 10 seconds. Each point represents the mean of 4 experiments.

reater influence on the et rate, whereas with two equential processes the reerse is true. If this is the xplanation of the unsual type of temperature ependence shown by the esults presented, the vo alternative processes ould have activation enrgies of the order of 8,000 cals mol^{-1} and ,000 cals mol^{-1} respecvely, the former being ate-limiting at 40°C, and he latter increasing in elative rate as the temerature is lowered. The ffect of stimulation on oth the shape and the osition of the Arrhenius lot may then be simply xplained by assuming hat it accelerates the rocess with the lower Q_{10} elative to the other, causng it to exert a greater nfluence on the net rate t a higher temperature.

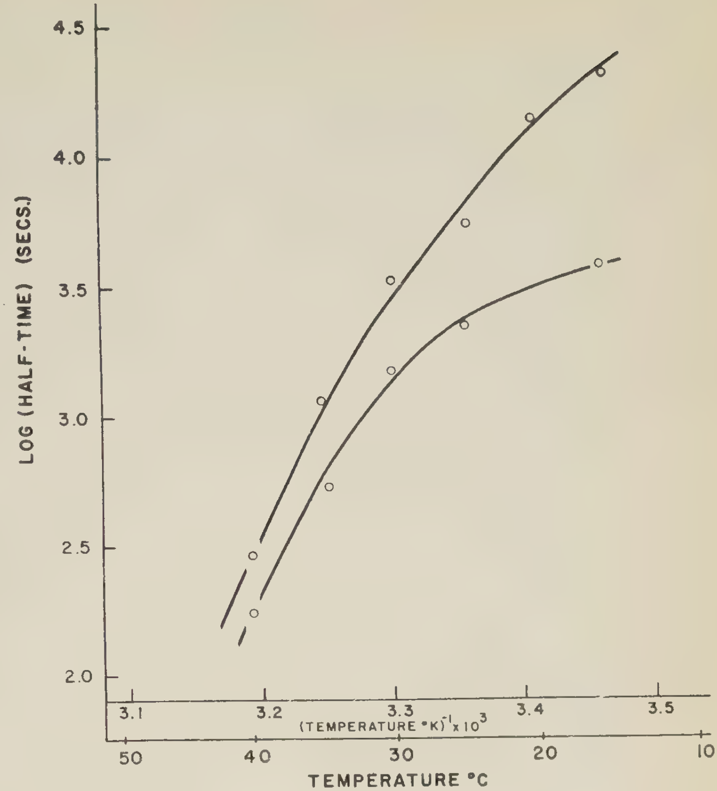

Fig. 3 Arrhenius plots obtained by taking vertical cross sections through figures 1 and 2 at a ryanodine concentration of 2.76×10^{-5} M. Lower curve, rat diaphragm stimulated every 10 seconds; upper curve, unstimulated rat diaphragm.

The slopes of the lines i figures 1 and 2 indicate hat the velocity of conracture is approximately roportional to the square root of the ryandine concentration, the exponent of the atter varying with the temperature beween 0.46 and 0.67 for the unstimulated eries and between 0.41 and 0.75 when he muscle is stimulated. Such a relation nay hold if there are two successive procsses which are of first order with respect o ryanodine and the product of the first rocess (Jenden and Haslett, '58), alhough in this case the slope should not all below 0.5[2]; it is also consistent with he general hypothesis outlined above. In complex system, the apparent reaction

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[2] This is based on the kinetic model described y the equations

$$\frac{dy}{dt} = k_1(y_0 - y) \qquad y(o) = 0$$

$$\frac{dz}{dt} = -k_2 y f(z) \qquad z(o) = z_0$$

here y is the concentration of the product of he first process, which may be diffusion into the cell; z is the concentration of the product of the second process and f (z) is any reasonable function of z, y_0 is the ryanodine concentration outside the cell and k_1 and k_2 are rate constants. The solution is

$$y = y_0(1 - e^{-k_1 t})$$

$$\int_{z_0}^{z} \frac{dz}{f(z)} = -\frac{k_2 y_0}{k_1}(k_1 t + e^{-k_1 t} - 1)$$

If t* is the time required for z to fall to any constant proportion of z_0,

$$S = \frac{d(\log t^*)}{d(\log y_0)} = \frac{1}{k_1 t^*} - \frac{1}{1 - e^{-k_1 t^*}}$$

so that

$$S \xrightarrow[k_1 t \to 0]{} -0.5$$

$$S \xrightarrow[k_1 t \to \infty]{} -1$$

$$-0.5 > S > -1$$

The slope of a logarithmic plot such as figures 1 and 2 will therefore vary between -0.5 and -1.0 and will be relatively insensitive to small changes in t, so that no significant deviation from a straight line relationship might be expected in the experimental data.

order is a better criterion for the identification of the rate-limiting process than the temperature coefficient (Glasstone, '46; Taylor, '31). The changing slopes of the lines in figures 1 and 2 may therefore reflect a shift in the relative importance of a zero order and a first order process as rate-limiting factors, the first order process exerting a greater influence on the net rate of contracture at higher temperatures.

One may tentatively conclude, therefore, that the rate-limiting step in the kinetics of ryanodine contracture may be a first order process with a very high temperature coefficient, but that under the experimental conditions used, some spontaneous (ryanodine independent) process with a much lower temperature coefficient ($\mu \sim 8{,}000$ cals mol^{-1}) occurs which favors the development of contracture. Stimulation of the muscle accelerates the latter process but may have little or no effect on the former. It seems quite reasonable to suppose that prolonged operation *in vitro* is associated with progressive changes in the muscle of a degenerative nature, and that these changes would be accelerated by contractile activity induced by electrical stimulation. It is perhaps pertinent that the Q_{10} of glycolysis, ATP turnover and creatine phosphate turnover during rigor mortis development in rabbit muscle is 1.63 (Bendall, '51), corresponding to an activation energy of 8700 cals mol^{-1}, in reasonable agreement with that of the ryanodine independent process.

The nature of the process limiting the rate of action of ryanodine remains in doubt. Ramsey ('56) suggested on the basis of an observed Q_{10} of 8.36[3] in frog muscle that a protein denaturation was involved. It is difficult to imagine that any substance in less than micromolar concentration can denature a protein by a direct interaction; such intense activity is usually associated with specific interference with a catalytic process. Denaturation, if it occurs, may therefore represent the end result rather than the primary effect of ryanodine.

A temperature coefficient of the order observed is by no means so high as to rule out processes other than protein denaturation. Tension development in glycerin extracted muscle fibers may have a heat of activation of 36,000 cals mol^{-1} or more (Brown, '57). A similar heat was found in intact heart muscle in the presence of high CO_2 concentrations. While ryanodine does not directly influence the shortening of myosin threads or glycerinated muscle fibers (Blum et al., '57) it is quite possible that this is the ultimate link affected as a result of a more immediate action of the drug.

It is also possible that diffusion through the muscle cell membrane is rate-limiting. Diffusion through the extracellular space is, of course, much too rapid to explain the kinetics and would have the Q_{10} of free diffusion (1.3–1.5). Diffusion through cell membranes is much slower and has a higher Q_{10}, the rate and Q_{10} being in general negatively correlated (Danielli, '41). The penetration of rat diaphragm muscle cells by pentoses has a Q_{10} of 2–3 (12), which is still far below that observed for ryanodine contracture; however, the ryanodine molecule is very large and also bears several free hydroxyl groups (Kelly et al., '51), so that a still higher Q_{10} may be expected. Extremely high temperature coefficients have been reported for cell permeability; the maximum uptake rate of 3-methyl glucose by Ehrlich ascites tumor cells is 10 times greater at 15°C than at 10°C ($Q_{10} \sim 100$) (Crane et al., '57). Diffusion into a cell would be a first order reversible process and therefore meets the requirements of the theory presented.

The curious relationship between size of rat and rate of ryanodine contracture of the isolated diaphragm requires comment. The existence of such a correlation has been confirmed in a separate series of experiments using rats between 40 and 200 gm. Contracture was up to 5 times slower in the smallest rats (Jenden and Scott unpublished). If the hypothesis outlined above is true, then the ryanodine dependent process is the one affected by animal size, since the correlation was only observed at higher temperatures and was only statistically significant at 40°C. It is unlikely that it is due to a more efficient oxygen supply in the thinner diaphragms of young animals, although anoxia is known to increase the sensitivity to ryanodine (Edwards and Flinker, '50; Ramsey et al., '50; Blum et al., '57). Oxygen diffusion in preparations taken from rats in the size range used has been shown to be adequate for normal metabolic needs (Creese et al., '58) and the QO_2 of rat diaphragm, unlike that of frog muscle (Edwards and Flinker, '50), is not greatly increased by ryanodine (Whalen et al., '58). It is possible that some subtle changes in the contractile system or its metabolic support occur during maturation of the animal; in this connection it is of interest that considerable changes in the phosphogen content of muscle have been reported during fetal development and maturation (Koschtojanz and Rjabinowskaja, '35; Rjabinovskaja, '35).

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[3] This corresponds to an activation energy of 35,000 cals mol^{-1} at 10–20°C. Ramsey erroneously gave 74,000 cals mol^{-1} for this figure.

LITERATURE CITED

Bendall, J. R. 1951 Shortening of rabbit muscles during rigor mortis: its relation to breakdown of adenosine triphosphate and creatine phosphate and to muscular contraction. J. Physiol., *114:* 71–88.

Blum, J. J., R. Creese, D. J. Jenden and N. W. Scholes 1957 The mechanism of action of ryanodine on skeletal muscle. J. Pharmacol., *121:* 477–486.

Booij, H. L., and H. P. Wolvekamp 1944 Catenary processes, master reactions and limiting factors. Bibl. Biotheor., *1:* 145–224.

Brown, D. E. S. 1957 Influence of Temperature on Biological Systems. Ed. by F. H. Johnson. Am. Physiol. Soc., Washington, D. C., pp. 83–110.

Crane, R. K., R. A. Field and C. F. Cori 1957 Studies of tissue permeability. I. The penetration of sugars into Ehrlich ascites tumor cells. J. Biol. Chem., *224:* 649–662.

Creese, R., N. W. Scholes and W. J. Whalen 1958 Resting potentials of diaphragm muscle after prolonged anoxia. J. Physiol., *140:* 301–317.

Danielli, J. F. 1941 Cell permeability and diffusion across the oil-water interface. Trans. Faraday Soc., *37:* 121–124.

Edwards, L. E., and M. L. Flinker 1950 Effect of length of a ryanodine-treated muscle on oxygen uptake. Proc. Soc. Exp. Biol. Med., *75:* 93–96.

Glasstone, S. 1946 Textbook of Physical Chemistry. Macmillan, London, chap. XIII, pp. 1044–1194.

Helmreich, E., and C. F. Cori 1957 Studies of tissue permeability. II. The distribution of pentoses between plasma and muscle. J. Biol. Chem., *224:* 663–679.

Jenden, D. J., and L. Scott 1957 Unpublished observations.

Jenden, D. J., and W. L. Haslett 1958 The relation between temperature, drug concentration and rate of contracture in ryanodine-treated muscle. J. Pharmacol., *122:* 34A (7803).

Johnson, F. H., H. Eyring and M. J. Polissar 1954 The Kinetic Basis of Molecular Biology. Wiley, New York, chap. 8, pp. 187–285.

Kelly, R. B., D. J. Wickingham and K. Wiesner 1951 The structure of ryanodine. I. Canad. J. Biochem. Physiol., *29:* 905–910.

Koschtojanz, K. L., and A. Rjabinowskaja 1935 Beitrag zur Physiologie des Skeletmuskels der Saugetiere auf verschiedenen Stadien ihrer individuellen Entwicklung. Arch. f. d. ges. Physiol., *325:* 416–421.

Ramsey, R. W., M. L. Flinker and L. E. Edwards 1950 Some actions of ryanodine on muscle. Oral presentation to Soc. of Gen. Physiol.

Ramsey, R. W. 1956 A study of the effects of the neutral alkaloid ryanodine on skeletal muscle and other tissues. ONR Technical Report No. 2, June 14. 1–8.

Rjabinovskaja, A. M. 1935 Weiteres über die ontogenese der Funktionen und des Stoffwechsels der Skeletmuskeln bei Säugetienen. Internat. Physiol. Cong., 15th, Proc. 533.

Stearn, A. E. 1949 Kinetics of biological reactions with special reference to enzymic processes. Adv. Enzymol., *9:* 25.

Taylor, H. S. 1931 Treatise on Physical Chemistry. 2nd edition, Chap. XV. D. Van Nostrand Co., Inc., New York, 4 vols.

Whalen, W. J., E. Dernberg and D. J. Jenden 1958 Interaction of stretch and ryanodine on oxygen uptake in the rat diaphragm. Am. J. Physiol., *194:* 359–362.

Sodium and Water Exchange in the Trout Egg

SUMNER M. KALMAN
Department of Pharmacology, Stanford University,
Stanford, California

The permeability properties of the freshly spawned Salmonid egg have intrigued biologists for a long time. Of particular interest is the process known as "water-hardening" which begins as soon as the eggs from the oviduct come into contact with brook water. This is associated with uptake of water, formation of a tough outer coating, the chorion, and the development of an inner cytoplasmic membrane which is believed to make the egg more or less impermeable to water for a long period. During this process a small amount of fluid and protein fills the space between chorion and plasma membrane (perivitelline space).

There has been some question as to whether electrolytes can penetrate the egg's yolk sac after water-hardening. Pumphrey ('31) suggested in a footnote that he had evidence for permeability to ions; eggs kept in tap water for some time seemed to lose electrolytes. Gray ('32) did some experiments to confirm his earlier ('20) conviction that the trout egg was normally impermeable to electrolytes when there was no demonstrable water permeability. In the same paper Gray ('32) made this cryptic remark, "The trout's egg is apparently completely insulated both in respect to salts and water — although an interchange of cations may occur with those in the environment."

Methods are now available to measure exchange rates of ions more precisely. The experiments to be described were set up for quantitative estimation of ion exchange in the unfertilized, water-hardened, trout egg. The idea that cations in particular may have access to the interior of cells by crossing the lipoprotein portion of the membrane in combination with a carrier molecule has formed an important part of current theories, particularly in regard to ion exchange in erythrocytes (Solomon, '52). If the aqueous pathways to the interior are indeed closed in the water-hardened trout egg this is an excellent system with which to test the carrier theory.

MATERIAL AND METHODS

Live rainbow trout (*Salmo gairdnerii*) were taken to the laboratory; eggs were gently expressed from the abdominal cavity into cool brook water and used on the same day. For a few experiments eggs freshly expressed from Lahonton cutthroat trout (*Salmo clarkii*) were transported to the laboratory in cold, aerated brook water. Tap water is a traditional medium for experiments on trout eggs because it usually contains a proper balance of important salts. The local tap water was highly toxic, probably due to its high chlorine content. As an alternative an artificial medium was used (suggested by an experiment of Gray in 1932); distilled water containing NaCl (0.52 M) and $CaCl_2$ (0.055 M). A few experiments done with plain brook water indicated that this solution had no adverse effects on the eggs. Both eggs and 10 ml of medium with appropriate tracers, were placed into a small, glass, Buchner type funnel with a fritted disc base. The vessel was fixed in a thermostat at 10°C, and a gentle stream of air was introduced through the fritted base. Individual eggs were removed at prescribed intervals. After rinsing in non-radioactive medium for a few seconds excess water was removed with the aid of moist filter paper. They were then placed into tared, capped vials and weighed. For determination of water content eggs were dried for 12 hours at 80°C and weighed again. Yolk samples were obtained by holding individual eggs between layers of gauze cloth and then introducing a needle tip into the center of the egg. Gentle suction with a ¼ ml syringe attached to a 30 gauge needle sufficed for this procedure.

The tracers used were $H_2{}^3O$, $Na^{22}Cl$ and NaI^{131}. The amounts employed related to convenience of counting, e.g., 2.5 millicuries of tritiated water and 80 microcuries of $Na^{22}Cl$ were used per 50 ml of medium for simultaneous exchange studies with these two tracers. For the iodide transfer experiment approximately 80 microcuries of NaI^{131} were added to 50 ml of medium. Both Na^{22} and I^{131} activities were measured in a well-type scintillation counter. The tritium content of each egg was estimated by adding 1 ml of distilled water to the egg, allowing it to stand overnight and then removing an aliquot for assay in the Packard Tri-Carb Liquid Scintillation Spectrometer. The method depends upon the assumption that this tracer substance will readily diffuse out of the egg when non-radioactive medium is added. Figure 1 demonstrates that this is indeed the case. The scintillation mixture consisted of 0.825 mg p-bis [2-(5-phenyloxazolyl)] 1-benzene, 106.5 mg 2,5-diphenyloxazole, 960 mg naphthalene, 1.5 ml xylene and 13.5 ml dioxane per sample This mixture was added to 250 μl of "diffusate" from each egg sample. Appropriate amounts of radioactive medium were diluted to 250 μl and counted in the same scintillation mixture.

For total sodium, potassium and chloride determinations, the eggs were frozen and then 1 ml of distilled water was added to prepare a "diffusate" as described in the preceding paragraph. A few determinations were done on homogenized eggs to establish that this technique did in fac yield a measurement of the total ion content of the egg. Sodium and potassium were determined with the Beckman flame photometer and chloride was assayed by means of the Aminco-Cotlove Titrator.

For comparative experiments eggs were placed in shallow beakers containing brook water and heated for 5 minutes at 33-35°C. I intended by this treatment to injure the egg membrane and to demonstrate concomitant changes in exchange rates.

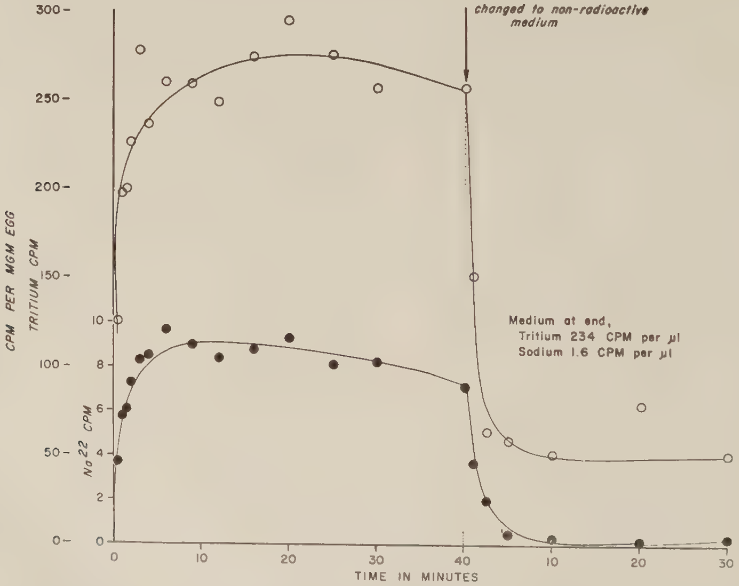

Fig. 1 The influx and efflux of tritium and radioactive $Na^{22}Cl$ by the unfertilized egg of the rainbow trout. Temperature, 10°C. The ordinate represents specific activity based on the wet weight of the eggs. The right section of the graph shows the efflux of the tracer materials when the medium was replaced by a non-radioactive salt solution of the same molarity (0.55 M NaCl, 0.05 M $CaCl_2$). Each point represents the mean for two eggs.

Two experiments were performed using O^{18}-labeled water. The medium for these experiments had the same salt concentration as indicated above, made up in water containing 1.5% excess O^{18} plus a trace amount of $Na^{22}Cl$. The O^{18} content of the eggs and medium was analyzed in a mass spectrometer at the Stanford Research Institute.

RESULTS

When fresh, unfertilized trout eggs were placed into a medium containing tritiated water and $Na^{22}Cl$ at 10°C there was an initial exchange of sodium and tritiated water with the egg content which was complete by about 5 minutes (fig. 2). The uptake rates of both substances were parallel, the absolute amount of tritium exchange being larger than that of the sodium. This difference was not always observed. At equilibrium the tritium content of each egg was equal to that contained in an amount of medium equivalent to about 30% of the egg water. The maximum deviation between individual eggs was 1.2%. Corresponding figures for the labeled sodium content of these same eggs were 19%, with a maximum deviation of 1.1%. Figure 2 (right) shows the same experiment performed with eggs that had previously been heated for three minutes at 35°C. There was a remarkable increase in total amount of exchange for each isotope; about 62% of egg water exchanged with the tritium in the medium, and about 68% of the sodium. The time required for equilibrium also appears to have increased.

In another pair of experiments the permeability of one batch of eggs was tested with respect to a medium containing tritiated water and radioactive sodium and in a separate experiment, to a medium containing O^{18}-labeled water and radioactive

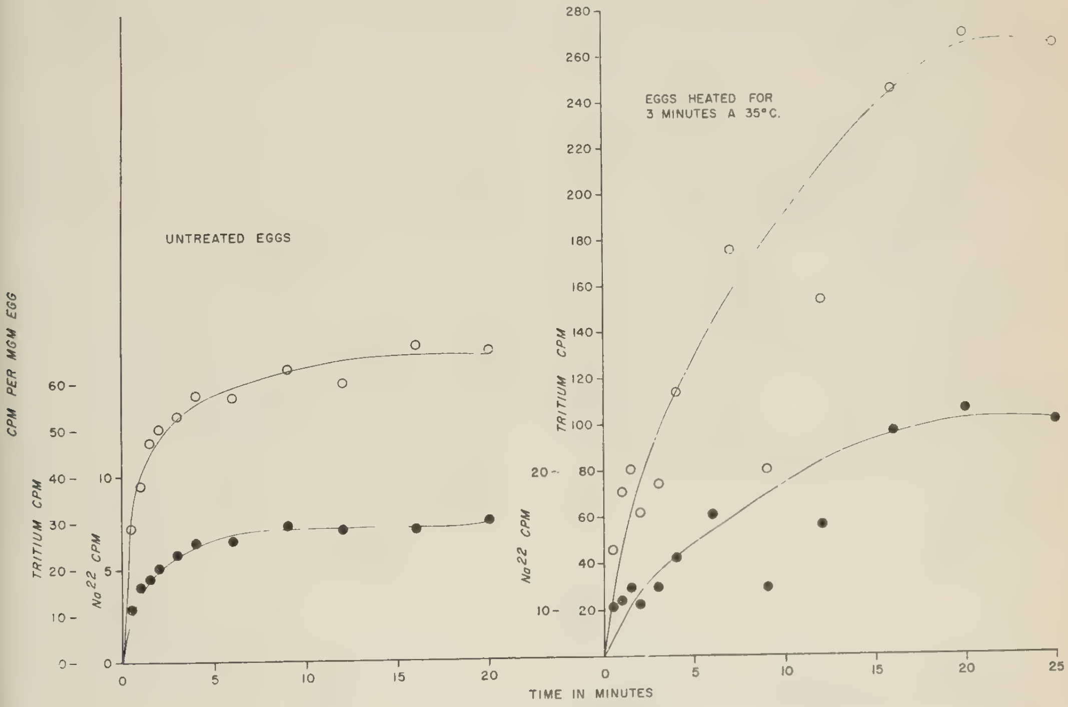

Fig. 2 The uptake of tritium and radioactive $Na^{22}Cl$ by the unfertilized egg of the rainbow trout. Temperature, 10°C. Medium, 0.55 M NaCl, 0.05 M $CaCl_2$. The graph at the left refers to untreated eggs. The graph to the right shows the effect of heating the eggs before placing them into the medium. Note that the ordinate scale has been changed in the graph on the right to get the results on the same page. Each point represents the mean for two eggs.

sodium. Results, presented in table 1,[1] show that sodium and tritium entered the egg in comparable amounts. This appeared to be the case also when sodium penetration was compared with O^{18} uptake, although the error in heavy oxygen estimation makes the agreement less elegant.

TABLE 1

Comparison of the exchange in trout eggs of (A) medium labeled with $Na^{22}Cl$ and $H_2{}^3O$ and (B) medium labeled with $Na^{22}Cl$ and H_2O^{18}

	Equilibrium values[1]	
	A—$Na^{22}Cl$ and $H_2{}^3O$	B—$Na^{22}Cl$ and H_2O^{18}
	% water equilibrated	*% water equilibrated*
Whole egg NaCl	27 (± 1.1)	29 (± 1.5)
H_2O	30 (± 1.2)	21 (± 7.0)
Egg yolk NaCl	9.5(± 2.0)	—
H_2O	13.8(± 4.9)	12.4(± 5.0)

[1] The figures represent the average per cent of egg water (in the case of the whole egg) equilibrated with medium; and per cent of yolk water (for egg yolk). The figures in parentheses represent *maximum* deviations between measurements based on individual egg activities.

A study was made with radioactive NaI^{131} in the medium to which the eggs were exposed. Figure 3 shows a result comparable to those obtained with sodium and tritium for the normal eggs. The heated eggs behave differently, showing a tendency to bind or trap iodide ions.

Results of total electrolyte analysis are present in table 2. The egg weight remained remarkably constant despite changes in electrolyte content. The potassium content of the egg did not change. Before exposure to the experimental medium the egg contained much more potassium than sodium. The gain in egg chloride was roughly parallel but somewhat greater than the sodium uptake.

DISCUSSION

The similarity in the exchange of tritiated water and radioactive NaCl with the trout eggs is of interest. The equilibration times correspond and the exchange rates are comparably affected by heating the eggs. In the case of the untreated egg there was the possibility that exchange was limited to the perivitelline space which lies beneath the chorion but external to the plasma membrane. It turned out that radioactivity was evenly distributed within the yolk substance, although its concentration was less than that of the whole egg. The higher level of activity found in the whole egg is probably due to the presence of the freely-exchangeable perivitelline fluid, about 10% of the egg volume.

These results again raised the question as to whether water exchange could occur between yolk and external medium. Krogh and Ussing ('37) and Prescott ('55) had investigated this problem by exposing eggs to a medium containing deuterium oxide. Krogh and Ussing noticed a permeability to water when unfertilized rainbow trout eggs were first placed into deuterium-labeled water. This permeability fell off to practically zero by 6 hours. Prescott studied individual salmon eggs and measured uptake of deuterium by a Cartesian diver method. He no-

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[1] The data in table 1 were calculated in the following way:

1. For these particular eggs the water content was 70%.

2. For Na^{22} and tritium the calculations were done in this way:

$$\frac{\text{total CPM per egg}}{\text{CPM per } \mu\text{l of medium}}$$

$= (X)\ \mu L$ of medium equilibrated with egg water

$$(100)\frac{(x)}{\text{egg } H_2O \text{ in } \mu L}$$

$= \%$ of egg water that equilibrated with medium

3. Example of calculation for O^{18} exchange; Given: total egg $H_2O = 48\ \mu l$. Let $x =$ egg water that equilibrated with medium, then $(48 - x) =$ egg water that did *not* equilibrate.

Results:

Non-labeled laboratory H_2O $= 0.00265$ atoms % O^{18}
Medium at equilibrium $= 0.01330$ atoms % O^{18}
Water distilled from egg at equilibrium $= 0.00475$ atoms % O^{18}

Then

$$(0.00265)(48 - x) + (0.01330)x = (48)(0.00475)$$
$$x = 9.5\ \mu l$$

Whence,

$$\frac{(9.5)}{(48)}\ 100 = \% \text{ egg } H_2O \text{ that equilibrated with medium}$$

4. The calculations for egg yolk values were done in an analogous manner, assuming yolk to contain 90% of total egg water. The perivitelline space is approximately 10% of the total egg volume (Bogucki, 1930).

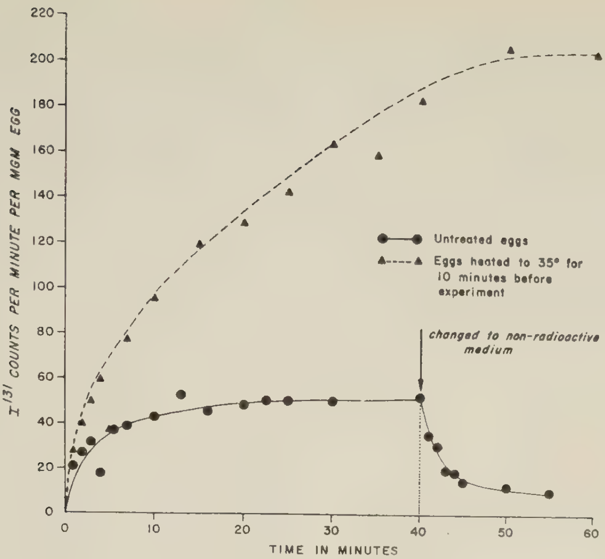

Fig. 3 The uptake of radioactive NaI^{131} by the unfertilized eggs of the rainbow trout. Temperature, 10°C. Medium, 0.55 M NaCl, 0.05 M $CaCl_2$. The lower curve represents uptake and release (see fig. 1) by the untreated eggs. The upper curve represents uptake (only) for heat-treated eggs. Each point represents the mean for two eggs.

ticed an initial uptake of deuterium when a water-hardened egg was first placed into the medium. This process was complete by two minutes and appeared to be confined to the perivitelline space. Neither of these papers presented results of egg yolk analysis. The results which I have obtained indicate an exchange between medium and yolk water at a time when there is no gross change in egg weight. The presence of radioactive tritium in egg yolk could be explained on the basis of penetration by the tritium ion. However, samples of egg yolk were obtained from eggs that had equilibrated with O^{18}-labeled medium. These samples contained heavy oxygen, although there was a lower concentration in yolk water than in whole egg water. This finding is strong evidence indeed for *water* exchange between yolk sac and medium.

The trout egg maintains an osmotic gradient between itself and the external medium. An absolute impermeability to water has been one generally accepted explanation of this phenomenon. Alternatives to this view include a "water pump" and mechanisms by which the egg can adjust its osmolarity in response to the environment and to changes induced by growth when the egg has been fertilized. Bogucki ('30) suggested that the egg maintains its integrity in the presence of a hypertonic medium by an equilibrium between colloidal materials in the perivitelline space and the force exerted by the solute in the medium. Presumably the egg could control the osmolarity of the colloidal materials in the perivitelline space. Svetlov ('28–'29) concluded that the plasma membrane plays a role in the regulation of osmotic pressure by furnishing a barrier to the penetration of electrolytes. But, he continued, this was only one side of the picture. The constant osmotic pressure observed during development must depend upon a continuous regulation of osmotically active material within the yolk substance since water uptake does occur during this period. My results would appear to support Svetlov's theory. However, it would be premature to claim that water and electrolyte exchange *always* occur. Different investigators have used different species of trout or salmon. They have handled their eggs in many different ways. Some have used fresh eggs; others have used stored eggs or eggs that were shipped from distant places. And, finally, it is clear that the permeability properties of the eggs depend to a large extent on the nature of the medium in which they are suspended (Loeb, '12).

Kao ('55a, b) has studied the properties of *Fundulus* (another teleost) eggs during activation (another term for water-hardening). His studies of pressure-volume relationships within the egg during exposure to hypertonic sucrose solutions indicate that there is an increase in the "incompressible fraction" of the egg that accompanies activation. This "incompressible fraction" behaves much like a compartment that does not exchange freely with the medium. These studies plus observations on increased electrical resistance of the plasma membrane after activation led Kao to conclude that water impermeability was achieved by a reduction in effective pore size. In agreement with me he sees no evidence for active transport of electrolytes in the Salmonid egg. Our views would coincide more exactly if we could agree that the water "impermeability" resulting from the hardening process is not absolute. Certainly one would not expect pores to be uniformly reduced in size by the mechanical events responsible for their compression.

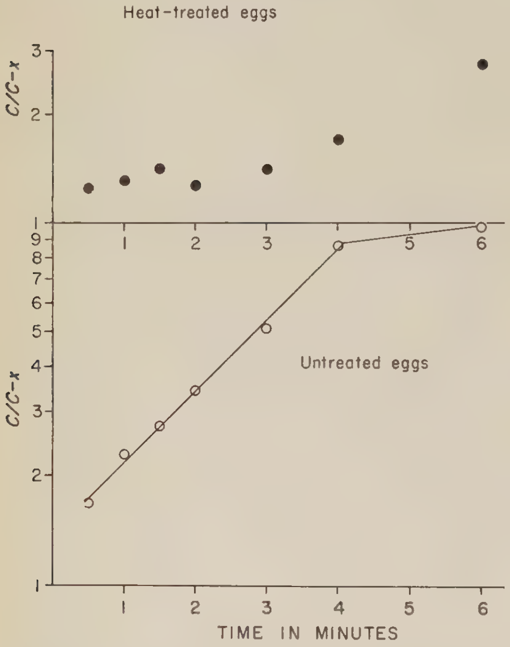

Fig. 4 Semilogarithmic plot of the uptake of radioactive $Na^{22}Cl$ by the trout egg. The data were derived from the same experiment presented in figure 1. The ordinate represents C/C-x plotted on a logarithmic axis; C refers to concentration of $Na^{22}Cl$ in the egg at equilibrium, x refers to concentration of $Na^{22}Cl$ in the egg at time, t. The complete equation is

$$\log \frac{C}{C\text{-}x} = \frac{KA}{V} \quad \frac{t}{2.303}$$

t = time in minutes
A = area available for diffusion
V = total volume of the egg
K = centimeters per unit of time
2.303 = the logarithmic conversion factor

It is assumed that A and V remain constant during the experiment.

The effect of heating the egg deserves special mention. It was employed, as indicated earlier, to injure the cytoplasmic membrane surrounding the yolk sac. A most impressive consequence of this treatment was the unexpected increase in the size of the exchangeable pool in the case of each ionic species studied. When plotted in accordance with an integrated form of Fick's diffusion equation (fig. 4) the sodium penetration into the untreated egg appeared to follow a first order process. This behavior is consistent with simple diffusion. The spherical shape of the eggs may be ignored where the radius is large (as in the trout egg) in comparison to atomic dimensions (Jacobs, '35). Figure 4 shows the same kind of plot for sodium penetration into a heated egg. The linear relation on semilogarithmic plot no longer obtains, indicating a more complex process, perhaps diffusion into multiple pools. The data for tritium yield similar curves, but there is much more scatter.

The penetration of iodide ion seems quite analogous to that of sodium ion and to the uptake of chloride. The obvious conclusion to be made is that exchange of electrolytes does occur between medium and yolk, and that this exchange is limited by the pool of exchangeable material within the egg. Heating the egg seems to enlarge this pool considerably.

The results of total electrolyte analysis (table 2) confirm the finding that ionic species have access to the interior of the

TABLE 2

Total electrolyte content of trout eggs as a function of time in hypertonic saline solution, 0.55 M NaCl and 0.05 M $CaCl_2$ at 10°C

Time	Egg weight[1]	Na[2]	K[2]	Cl[2]
min.	*mg*	*m.e./kg*	*m.e./kg*	*m.e./kg*
0	68.4	15.2	45.2	49.6
0.5	68.7	55.1	51.4	153.3
1.0	65.8	70.3	53.0	165.8
1.5	67.7	66.2	49.0	125.6
2	66.7	75.7	51.1	134.2
3	70.6	77.5	45.5	133.2
4	68.2	90.5	50.5	140.1
6	68.8	93.8	49.7	159.9
9	70.0	92.0	53.5	149.3
12	68.8	101.9	53.0	158.4
16	67.0	96.1	48.0	167.6
20	67.0	98.4	48.9	155.6
25	68.6	96.1	43.9	157.8
30	65.8	104.4	46.0	169.6
30	yolk[3]	53.0	65.34	104.8
30	medium[4]	440	none	437

[1] Egg weight, each figure represents the mean value for the net weight of two eggs.
[2] Na, K, and Cl are expressed as m.e./kg wet egg weight.
[3] Yolk represents a combined sample obtained from 5 eggs.
[4] Medium, electrolyte concentration expressed in m.e./l.

egg.[2] They indicate further that some mechanism must operate to offset the striking changes in osmotically active materials that can be induced by alteration of the environment. The compensatory response which must operate to maintain a constant osmotic pressure in the yolk substance in the face of a large increase in sodium chloride concentration is not apparent. It is apparent that no concomitant loss of potassium, or potassium chloride, occurs. In fact the stability of egg potassium adds another enigma to our list.

SUMMARY

Experiments with fresh, water-hardened trout eggs showed a definite, but limited, permeability to anions and cations and to water on the part of the egg's yolk sac at a time when no perceptible change in egg weight occurred. The conclusions were based primarily upon the presence within the egg yolk of tracer materials originally placed into the medium. Another finding

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[2] It can be shown that the gain in total sodium represents increments distributed between perivitelline space and yolk sac in a well-defined manner. The amount of sodium in brook water (0.071 m.e./l) is so small compared to that in the egg that the sodium in the egg at zero time may be considered to be in the yolk. The following calculations are based on the assumption that there is essentially free exchange between medium and perivitelline space. There is general agreement about this in the literature.

The mean egg weight is 68.2 mg. Assume 90%, 61.38 mg, is yolk. Then 10%, 6.82 mg, is perivitelline space.

Total egg sodium at equilibrium,

$(68.2)(0.0993) = 6.77\ \mu e.$

($\mu e.$ = microequivalents.)

0.0993 is the mean value in $\mu e./mg$ for the 5 samples, 12 minutes to 30 minutes, inclusive.

Total egg sodium at start,

$(68.2)(0.0152) = 1.04\ \mu e.$

Difference; gain in total sodium = 5.73 $\mu e./egg$

Yolk at equilibrium,

$(61.38)(0.053) = 3.25\ \mu e.$

Yolk at start = 1.04 $\mu e.$

Gain in yolk sodium = 3.25 − 1.04 = 2.21 $\mu e.$ (A)

Perivitelline sodium at end is essentially equivalent to gain in perivitelline sodium.

$(6.82)(0.440) = 3.00\ \mu e.$ (B)

Hence,

Calculated gain in sodium (A + B) = 5.21 $\mu e./egg$

Actual gain in sodium = 5.74 $\mu e./egg$

Thus, the uptake of sodium may be accounted for on the basis of the following description of the egg: The egg substance is divided between a perivitelline space which exchanges freely with the environment, and a yolk sac which *does* exchange with the environment, but to a limited extent. For chloride the calculated gain (A + B) is 6.24 $\mu e.$, whereas the actual gain in total chloride was 7.65 $\mu e.$

of interest was the observation that heating the eggs before the experiment caused a large increase in the size of the exchangeable sodium chloride pool. Ancillary experiments included studies of total electrolyte content and, also, penetration of radioactive iodide ion into the egg.

ACKNOWLEDGMENTS

The author would like to express his thanks to Professors Frederick Fuhrman and Avram Goldstein for reviewing this manuscript, and to Professor Ernst Bárány for helpful suggestions. The experimental work was supported by a U. S. Public Health Service Grant. The cooperation of Mr. Earl C. Leitritz and the staff of the California Department of Fish and Game was much appreciated.

LITERATURE CITED

Bogucki, M. 1930 Recherches sur la perméabilité des membranes et sur la pression osmotique des oeufs des salmonides. Protoplasma, *9:* 345–369.

Gray, J. 1920 The relation of the animal cell to electrolytes. J. Physiol., *53:* 308–319.

——— 1932 The osmotic properties of the eggs of the trout (*Salmo fario*). J. Exp. Biol., *9:* 277–299.

Jacobs, M. H. 1935 Diffusion processes. Ergebnisse der Biologie, XII. Springer Verlag, Berlin, pp. 1–160.

Kao, C. Y. 1955a Pressure-volume relationship of the *Fundulus* egg in sea water and in sucrose. J. Gen. Physiol., *40:* 91–105.

——— 1955b Changing electrical constants of the *Fundulus* egg plasma membrane. Ibid., *40:* 107–119.

Krogh, A., and H. Ussing 1937 A note on the permeability of trout eggs to D_2O and H_2O. J. Exp. Biol., *14:* 35–37.

Loeb, J. 1912 Antagonistic action of electrolytes and permeability of the cell membrane. Science, *36:* 637–639.

Prescott, D. M. 1955 Effect of activation on the water permeability of salmon eggs. J. Cell. and Comp. Physiol., *45:* 1–12.

Pumphrey, R. J. 1931 The potential difference across the surface bounding the unfertilized egg of the brown trout. Proc. Roy. Soc. London, Series B., *108:* 511–521.

Solomon, A. K. 1952 The permeability of the human erythrocyte to sodium and potassium. J. Gen. Physiol., *36:* 57–110.

Svetlov, P. 1928–1929 Entwicklungsphysiologische Beobachtungen an Forelleneiern. Roux's Archive für Entwicklungsmechanik der Organismen, *114:* 771–785.

Estimation of Thyroid Secretion Rate of Rainbow Trout Using Radioactive Iodine[1,2]

JACK R. HOFFERT[3] AND P. O. FROMM
Department of Physiology and Pharmacology, Michigan State University, East Lansing, Michigan

The literature dealing with the iodine metabolism of the teleostean thyroid has been summarized by Pickford and Atz ('57) and Hoar ('57). The available data indicate that iodine metabolism in teleosts is similar to that in higher vertebrates. By use of chromatograms, Gorbman and Berg ('55) were able to identify monoiodotyrosine, diiodotryrosine and thyroxine in *Fundulus* but no triiodothyronine appeared to be present. The iodine compounds, their order of appearance in the chromatograms, and their relative proportions did not differ in any significant way from the thyroxineogenic cycle of mammals. The above-named iodine compounds including triiodothyronine were found in a mudskipper, *Periophthalmus koelreuteri* by Leloup ('56).

There have been many varied means by which workers have tried to estimate the activity of the thyroid. According to histological criteria, active follicles are described as having increased colloid material, taller cells with rounded nuclei, increased cytoplasm and large dark nucleoli; the lack of these characteristics indicates inactive follicles. It has been shown by Nadler and Leblond ('55) that differences in colloid diameter when statistically examined could be shown to be identical to cross-sections taken at different planes through a spherical follicle. Changes in the histological appearance of a given gland may show whether or not the tissue is being stimulated but cannot be used as a means of estimating thyroid activity in terms of the amount of hormone being released by the thyroid.

Since radioiodine has become readily available, more investigators have been determining the I^{131} uptake and output rates as a means of estimating thyroid activity. The rate and amount of I^{131} uptake by the thyroid as well as the output rate are affected by various factors such as the size of the iodine pool, amount of recycling, and the rate of excretion of iodine by the organism. Thus, these measurements may be used to indicate relative functional activity but they do not indicate the actual rate at which thyroxine is being produced by the thyroid tissue.

Perry ('51) using groups of rats injected with different amounts of thyroxine, showed that inhibition of thyroidal I^{131} output was proportional to the amount of thyroxine given. Reineke and Singh ('55) investigated the effects of increased dosage of thyroxine to individual animals and also found that thyroidal I^{131} output was decreased proportional to the amount of thyroxine given. Based on these results a method for the estimation of thyroid secretion rates was proposed by these investigators. Since the pattern of iodine metabolism and control of the teleostean thyroid is similar to that of higher vertebrates, it is believed that this method should be satisfactory for the determination of thyroid activity in trout.

The actual rate of thyroid hormone formation in poikilothermous vertebrates has, to our knowledge, never been measured. The present work was undertaken to obtain an estimate of the thyroid secretion rate of rainbow trout and to deter-

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[2] The results of these experiments have appeared in abstract form in The Physiologist, *2:* p. 43 (1959).

[3] This paper is based on a thesis submitted in partial fulfillment of the requirements for the Master of Science degree in the College of Science and Arts, Michigan State University.

mine how temperature affects this rate. Experiments were also performed on different age groups of similar size.

MATERIALS AND METHODS

Rainbow trout, *Salmo gairdnerii,* with an average weight of 14.0 gm (standard deviation, 3.4 gm) were obtained from the Wolf Lake hatchery which is operated by the Michigan Department of Conservation. They were kept under conditions as described by Schiffman and Fromm ('59) and fed dried trout pellets which contained 2% iodized salt.

The fish to be injected were first anesthetized with MS-222. All injections were made using a syringe connected to a microburet and fitted with a 27-gauge needle. Solutions were injected into the pleuroperitoneal cavity approximately 2 cm anterior to the pelvic fins and the volume injected was 0.2 cm^3 or less.

Carrier-free radioiodine as NaI^{131} was obtained from the Oak Ridge National Laboratory. The crystalline l-thyroxine used was supplied by Glaxo Laboratories, Greenford Middlesex, England, and was purified by Dr. E. P. Reineke.

Three days after injection of 12 μc. of I^{131} the *in vivo* activity in the head region of individual fish was measured at constant geometry using a setup similar to that described by Fromm and Reineke ('56) but with improved shielding and better collimation. A second *in vivo* count was made of each fish. This animal background count of an area posterior to the transverse septum included activity in

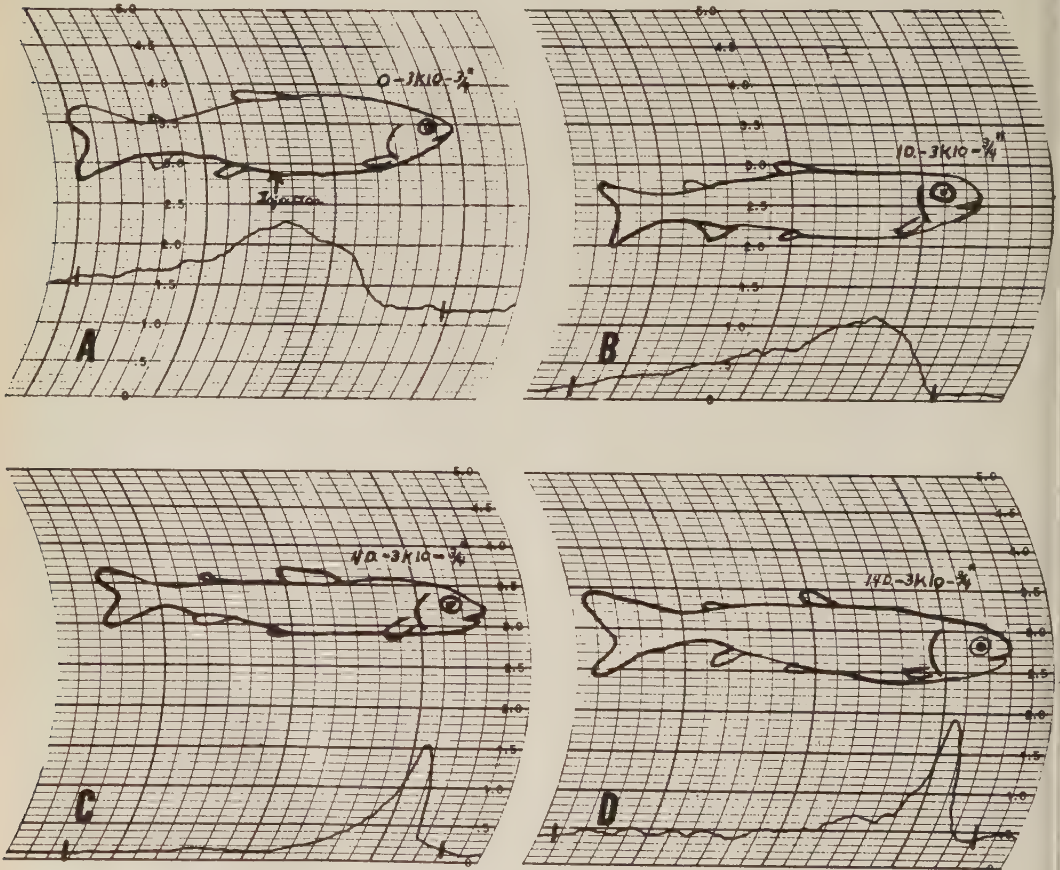

Fig. 1 Gross radioactive map of rainbow trout after injection of radioiodine. A, map of dead fish injected with I^{131}. B, C, and D, maps of fish 1, 4 and 14 days after injection respectively.

such organs as the stomach, liver, kidney, gonads, and part of the intestine, as well as the I^{131} in the blood and pleuroperitoneal cavity.

Due to the inability of the counting apparatus to count only thyroid I^{131} activity, a certain per cent of the animal background was included in the total activity of the *in vivo* thyroid measurements. By comparing *in vivo* counts and counts on isolated lower jaws it was found that subtracting one-half of the total body background count resulted in complete correction of thyroidal area counts made three days post-injection and thereafter. However, data obtained during the first two days following injection were not completely corrected for body background by this procedure. It should be mentioned here that body background activity decreased rapidly during three to 4 days following injection of I^{131} and the importance of making correction becomes relatively less as the time interval between injection and measurement increases.

Oxygen consumption measurements were made using equipment as described by Fromm ('58) and records of opercular rate were made from visual observation.

RESULTS

Gross distribution of injected I^{131}

For this study one fish was sacrificed and then injected with I^{131}. Five other fish were injected with I^{131} and then sacrificed at varying time intervals. A "gross radioactive map" was made of each fish using a Model C-100 Actigraph Strip Feeder (Nuclear-Chicago). The fish, after being sacrificed, were placed on an aluminum strip table and fed over a slit collimator while a record of the counting rate was made. The results shown in figure 1 indicate that during the first few days following injection the greatest concentration of I^{131} gradually shifts to the thyroidal area. After 4 days there was no further change in the position of maximal activity.

Output of I^{131} from thyroidal area

The activity in the head region of 8 fish was determined over a period of 8 days following injection of 12 μc. of I^{131}. Counts

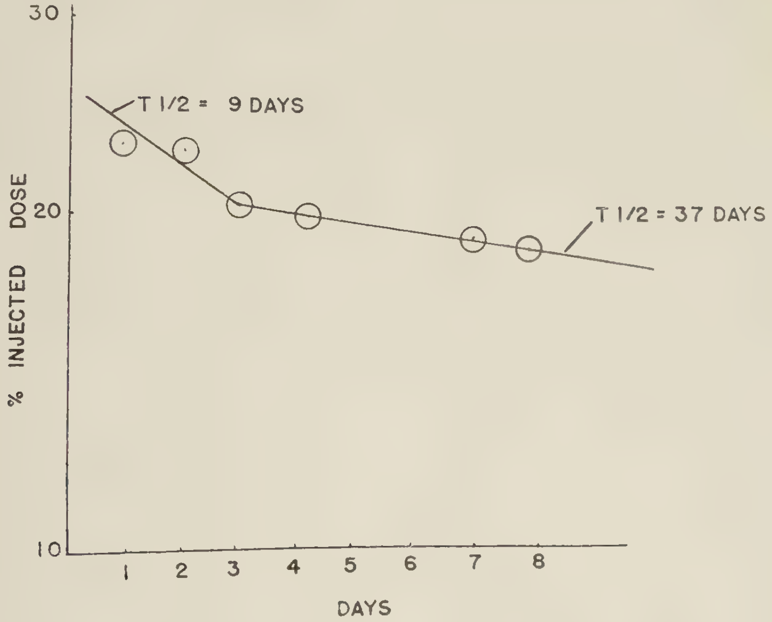

Fig. 2 Semi-log plot of output of radioiodine from the thyroid of rainbow trout. The activity, expressed as per cent of injected dose corrected for animal background, is plotted versus time in days after I^{131} injection.

of body background taken during this same period showed a very rapid loss during the first 4 days following injection ($T\frac{1}{2} = 2.5$ days). The body background thereafter remained at a value of approximately 4½% of the injected dose.

The corrected values for thyroidal I^{131} activity are plotted in figure 2. The data show that during the first 3 to 4 days following injection there is a rapid loss of I^{131} from the thyroidal area. This fast component ($T\frac{1}{2} = 9$ days) shown in figure 2 is due to incomplete correction for body background activity. The slow component, $T\frac{1}{2} = 37$ days, represents a true rate of release of I^{131} from the thyroidal area.

Thyroid secretion rates

Secretion rate determinations were made on both one- and two-year-old trout of approximately the same size and kept at 13°C. Rates were also determined for one-year-old trout which had been cooled from 13°C to 3°C in 24 hours and maintained for one week at the lower temperature prior to the start of the experiment.

An *in vivo* count was taken three days after I^{131} injection and every two days thereafter. Starting with the fifth day, a series of increasing thyroxine injections were given; the dose (in μg/100 gm body weight) was increased on the day of counting after the count had been made. Figure 3 shows the response of 11 one-year-old trout kept at 13°C. The solid line used to estimate 100% previous count and the secretion rates was fitted by the method of least squares. The dotted lines (Sy) are plots of the standard deviations about the fitted line. At the intercept of the Sy lines with the 100% previous count line, perpendiculars were dropped

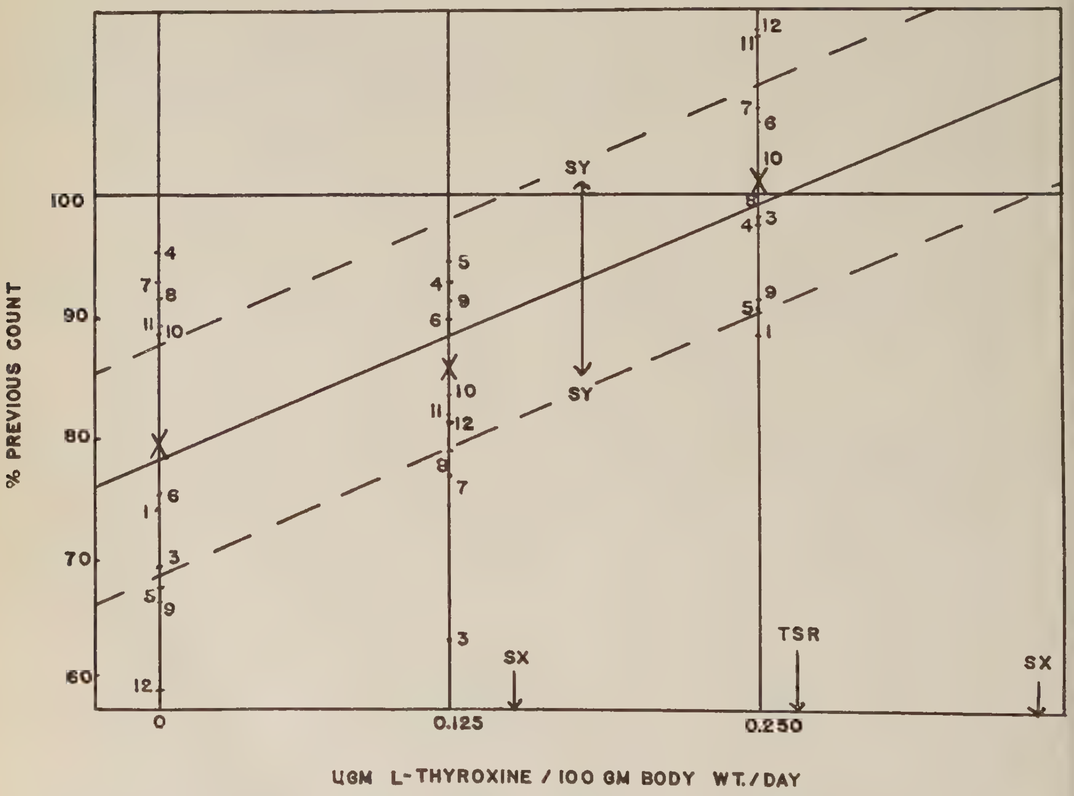

Fig. 3 Response of 11 one-year-old rainbow trout to thyroxine injections. See text for details. Abscissa: μgm l-thyroxine/100 gm body weight/day. Ordinate: radioactivity of thyroid as per cent of previous count.

TABLE 1

Mean values of thyroid secretion rate, oxygen consumption and opercular rate of one-year-old rainbow trout kept at 3° and 13°C along with thyroid secretion rate of two-year-old fish kept at 13°C

Group	No. of fish	Age	Temp.	Month data obtained	Thyroid secretion rate	Oxygen cons.	Opercular rate
		years	°C		*µg l-thyroxine/ 100 gm/day*	*(ml/gm/hr)*	*(number/min)*
I	18	2	13	Aug.	0.303 ± 0.082[1]	—	—
II	18	1	13	Sept.–Oct.	0.244 ± 0.035	0.087	177
III	10	1	3	Oct.	0.139 ± 0.017	0.040	61

[1] Standard error.

from the "100% line" to the x axis. The distance from these intercepts with the x axis (2 × Sx) is equivalent to two standard deviations of the estimated thyroid secretion rate. Therefore, the standard deviation of the secretion rate is given by Sx. Sx will be valid if Sy is a measure of the true variance about the fitted line. Since two-thirds of the observations fall within one standard deviation of the fitted line, the assumption that Sy is a valid measure of the standard deviation is strengthened. From the standard deviation (Sx) the standard error of the thyroid secretion rate was calculated. With these data, tests for significant differences were made using the t-test.

The mean thyroid secretion rates for yearling trout kept at 13° and 3°C were 0.244 and 0.139 µg l-thyroxine/100 gm/day respectively. No significant differences in thyroid secretion rate occurred between the different age groups kept at the same temperature. The secretion rates of the fish kept at 3°C were significantly lower than both the two-year-old fish ($p = 0.05$) and one-year-old fish ($p = 0.01$) kept at 13°C.

DISCUSSION

No data on thyroid secretion rate of cold-blooded vertebrates were found in the literature with which we can compare the results presented here for rainbow trout. Reineke ('59) has summarized the values for homoiothermic animals which have been obtained using a similar technique as that used with the trout. Expressing the results as $\delta/kg^{0.73}$/day, mice, rats, mink, sheep, and goats have secretion rates varying from 10 to 19 times greater than that of trout. It is well known that homoiotherms have a much higher basal metabolism than poikilotherms; hence it is not surprising that the thyroid secretion rate for trout is far below that of warm-blooded animals. This relationship would be expected even though no clear cut demonstration has been given concerning the relation between the piscine thyroid and oxidative metabolism in fish.

In order to get reliable estimates of the thyroidal I^{131} content in fish, it is necessary to correct for accumulation and output of nonthyroidal radioiodine. Our results show that this body background activity has a half-life of approximately 2.5 days, a value which compares favorably with the output rate of I^{131} from the head region of thyroidectomized trout ($T\frac{1}{2} = 1.8$ days) reported by Fromm and Reineke ('56). Data on release of radioiodine from the head region of brown trout during 72 to 96 hours after administration reported by Swift ('55, '59) is of questionable value for the estimation of thyroid activity since no correction for body background was made. Factors other than thyroidal release (e.g., rate of excretion of iodide) may have a greater influence on the rate of loss of I^{131} from the head region than thyroidal activity itself.

Based on histological criteria alone, Olivereau ('55a) has found no difference in thyroid activity in carp, tench, eels, mullets, and sharks studied at low (5° to 14°C) and high (20° to 23.5°C) temperatures. These fish apparently have a relatively constant level of thyroid activity which is independent of water temperature. Using both histological criteria and radioautographic techniques she has reported (Olivereau, '55b) a greater thyroid activity in rainbow trout kept at 9° to 12°C than in fish kept at 20°C.

In contrast to these data our results show that rainbow trout acclimated to 3°C have thyroid secretion rates significantly below those of fish kept at 13°C. Q_{10} values for the 10° range of temperature were calculated from mean values presented in table 1. On the basis of Q_{10} values of 2.17, 1.97, and 1.75 for oxygen consumption, opercular rate and thyroid secretion rate, respectively, one would hesitate to conclude that these values indicate that thyroid activity is relatively greater at 3°C than either opercular rate or oxygen consumption. It seems rather unlikely that the thyroid plays an essential role in the control of oxidative metabolism of the trout at low temperatures or that the thyroid is concerned in a temperature-compensating mechanism.

In agreement with the trout data by Olivereau cited above are the conclusions reached by Swift ('59). As previously stated, the data presented by Swift on thyroid activity are of questionable value. Nevertheless, he reports that with the exception of a short burst of activity found in July, the thyroid activity of yearling brown trout was inversely proportional to the water temperature. If one treats the data for three-year-old brown trout (Swift, '55) as the data were handled in the 1959 paper it appears that, with the exception of data for February and July, thyroidal activity was *directly* proportional to water temperature!

With regard to thyroid activity of fishes of different ages few data are available. Swift ('59) reports that three-year-old brown trout had much more active thyroids than the yearling fish. This difference in activity was believed to be correlated with gonad maturation in the older fish. Our results show no differences between one- and two-year-old rainbow trout of the same size.

CONCLUSIONS

1. Thyroid secretion rates of *Salmo gairdnerii* were determined using a technique which has been successfully applied to homoiothermic animals. The secretion rate of trout was found to be some 10 to 19 times below values for homoiothermic animals.
2. No significant difference in the thyroid secretion rate of one- and two-year-old fish maintained at 13°C was found.
3. One-year-old fish acclimated to 3°C had thyroid secretion rates significantly lower than those of fish of both age groups kept at 13°C.
4. The need for making body background corrections in studies of output of radioiodine from the thyroid of fish is discussed.
5. It is concluded that the thyroid of rainbow trout has little or no influence on the oxidative metabolism at low temperature and it appears doubtful that the trout thyroid is involved in a temperature compensating mechanism as has been previously postulated.

ACKNOWLEDGMENT

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LITERATURE CITED

Fromm, P. O. 1958 A method for measuring the oxygen consumption of fish. Prog. Fish-Cult., *20:* 137–139.

Fromm, P. O., and E. P. Reineke 1956 Some aspects of thyroid physiology of rainbow trout. J. Cell. and Comp. Physiol., *48:* 393–404.

Gorbman, A., and O. Berg 1955 Thyroid function in the fishes *Fundulus heteroclitus*, *F. majalis* and *F. diaphanus*. Endocrinology, *56:* 86–92.

Hoar, W. S. 1957 In: Physiology of Fishes. Vol. I, Metabolism, edited by M. E. Brown. Academic Press, Inc., New York.

Leloup, J. 1956 Contribution à L'étude du fonctionnement thyroïdien d'un téléostéen amphibiotique: *Periophthalmus koelreuteri*. C. R. Acad. Sci., Paris, *242:* 1765–1767.

Nadler, N. R., and C. P. Leblond 1955 The site and rate of formation of thyroid hormone. Brookhaven Symp. Biol., *7:* 40–58.

Olivereau, M. 1955a Influence de la température sur l'histologie thyroidïenne de divers téléostéens. Bull. Soc. Zool. Fr., *80:* 43–52.

——— 1955b Influence de la température sur la fonctionnement thyroidïenne de la truite étudié â l'aide de l'iode radioactif. C. R. Soc. Biol., Paris, *149:* 536–539.

Perry, W. F. 1951 A method for measuring thyroid hormone secretion in the rat with its application to the bioassay of thyroid extracts. Endocrinology, *48:* 643–650.

Pickford, G. E., and J. W. Atz 1957 The physiology of the pituitary gland of fishes. New York Zool. Soc., New York.

Reineke, E. P. 1959 Thyroid function in several species of animals as related to environment and body size. Conf. on Radioactive Isotopes in Agriculture, Stillwater, Okla. Apr. 2–3, 1959. U.S. Atomic Energy Commission, in press.

Reineke, E. P., and O. H. Singh 1955 Estimation of thyroid hormone secretion rate of intact rats. Proc. Soc. Exp. Biol. Med., *88:* 203–207.

Schiffman, R. H., and P. O. Fromm 1959 Measurement of some physiological parameters in rainbow trout (*Salmo gairdnerii*). Canad. J. Zool., *37:* 25–32.

Swift, D. R. 1955 Seasonal variations in the growth rate, thyroid gland activity and food reserves of brown trout (*Salmo trutta* Linn.) J. Exp. Biol., *32:* 751–764.

——— 1959 Seasonal variation in the activity of the thyroid gland of yearling brown trout, Salmo trutta Linn. Ibid., *36:* 120–125.

Initiation of Spike Potentials in Contact Chemosensory Hairs of Insects

I. THE GENERATION SITE OF THE RECORDED SPIKE POTENTIALS[1]

HIDEKI TATEDA[2] AND HIROMICHI MORITA
Department of Biology, Faculty of Science, Kyushu University, Fukuoka, Japan

The electrical activity of the chemosensory hairs of insects upon the chemical stimulation has been recorded by some authors (Hodgson and Roeder, '56; Morita et al., '57; Wolbarsht and Dethier, '58). Morita et al. reported that the spike potentials were recorded from the chemosensory hairs of some insects as an increase in positivity at the tip of the hair with respect to its proximal part. This was supported by Hodgson ('58) and by Wolbarsht ('58). If it is supposed that the recorded spike potentials occur at the tip of the hair, it must be concluded that the spike potentials are picked up by the extracellular electrodes as an increase in positivity with respect to the resting region. However, some authors presumed that the impulses were generated in the cell body (Dethier, '56; Hodgson, '58; Wolbarsht, '58). The present investigation was undertaken to solve this problem on the labellar chemosensory hairs of the fly, and ascertained that the generation site of chemosensory spikes was at the proximal part of the hair.

MATERIAL AND METHODS

The fly, *Lucilia caeser*, collected in the field was used as the experimental material. The experiments were carried out at room temperatures during November and December, 1957.

The methods used in these experiments were similar to those used by Hodgson and Roeder ('56). The proboscis of a fly was isolated from the head by a forceps and a platinum wire was inserted into it. This platinum wire was fixed on a rubber block and was used as the indifferent electrode. The active electrode consisted of a glass capillary, about 50 μ in tip diameter, containing M/8 NaCl solution and a platinum wire which was dipped into the solution. The active lead was connected to the grid of the pre-amplifier which had no grid resistor and the grid current was less than 10^{-11} amperes. The active electrode was moved with the aid of a micromanipulator and the solution contained in the capillary was brought into contact with the tip of the hair. The pre-amplifier was followed by a R-C coupled amplifier and a cathode-ray oscilloscope. The electrical activity of the sensory hair was recorded on a tape recorder. Tapes were subsequently replayed into the oscilloscope for photographic recording. A calibration or test pulse was generated in a low resistance pulse generator inserted between the indifferent electrode and the ground.

Sometimes, another method was employed for recording the spike potentials. In this case, the active electrode was kept in contact with the hair wall at the middle part of the hair. When the suitable hair was selected, the spike potentials could be picked up by contacting only this electrode with the hair wall. In order to give stimulation to the hair, the test solution contained in another capillary was brought into contact with the tip of the hair.

RESULTS

Spike potentials recorded from the cut hair remaining on the labellum

If the recorded spike potentials were actually initiated at the tip of the hair,

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[1] Contributions from the Department of Biology, Faculty of Science, Kyushu University, No. 71. Aided in part by the Scientific Research Fund from the Ministry of Education.

[2] Present address is the Department of the Biological Sciences, Florida State University, Tallahassee, Florida, U. S. A.

they might be recorded from an isolated distal part of a hair whose tip was kept intact. In order to record the spike potentials from the isolated hair tip, the distal part of the labellar chemosensory hair was cut off from its proximal part with a steel forceps. The forceps had been connected with the indifferent electrode, and the isolated part of the hair was supported with it. Then, the tip of the hair was brought into contact with the NaCl solution contained in the capillary electrode. Impulses were never obtained from the isolated part of the hair though they had been recorded with the usual method before cutting, even when the isolated hair was long enough for avoiding the injury effect on the tip. On the other hand, when the capillary electrode was brought into contact with the cut end of the hair remaining on the labellum, avoiding mechanical stimuli, the electrical activity was often recorded from it (fig. 1, B). The low frequency of impulses was maintained without a decay during the whole course of contact with the active electrode. Therefore, it can be assumed that the spike potentials which were recorded from the cut end of the hair stump were not generated by chemical stimulation, but by an injury effect or other causes. There was another kind of spike potential evoked by mechanical stimulation alone. In some hairs, the spike potentials were not recorded from the stump on the labellum by contact with the active electrode, but they appeared when the stump was bent. The polarity of two kinds of spike potentials was the same as that obtained from the intact hair.

To determine the origin of the spike potentials, the distal part of a chemosensory hair was removed successively, and the polarity and heights of the spike potentials from the cut hair on the labellum were compared with those from the intact hair. The spikes recorded from the stump as a response to mechanical stimulation were almost the same in size as those from the intact hair. Since the calibration pulse was then recorded as a larger deflection than in the intact hair, because of the decrease in resistance due to the removal of the hair tip, the calibrated heights of the spikes were smaller than those before the removal. Figure 2 shows the relation between the spike height and the length of the hair stump from which both kinds of impulses were recorded. It can be seen from this figure that the spike heights of both kinds of impulses were decreased according as the length of the stump of the sensory hair on the labellum was shortened by cutting off the distal part. The height of the spike occurring with mechanical stimuli in the stump was about 80% of that in the intact hair while that without mechanical stimuli was reduced to a half of that in the intact

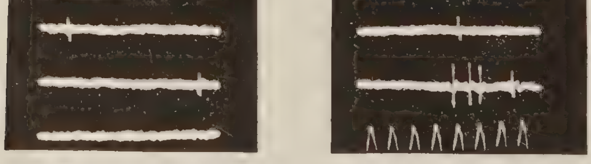

Fig. 1 Records of the impulses from the labellar chemosensory hair before and after the removal of its tip. The tip or the cut end of the hair was in contact with ⅛ M NaCl solution contained in the capillary of the active electrode. A, spike potentials from the intact hair. Large spikes occurred by mechanical stimulation given for a short period. Small spikes occurred without mechanical stimulation. B, spike from the cut hair remaining on the labellum. Large spikes occurred by mechanical stimulation. Small spikes occurred without mechanical stimulation. Time, 60 cycles/second. Retouched photographs.

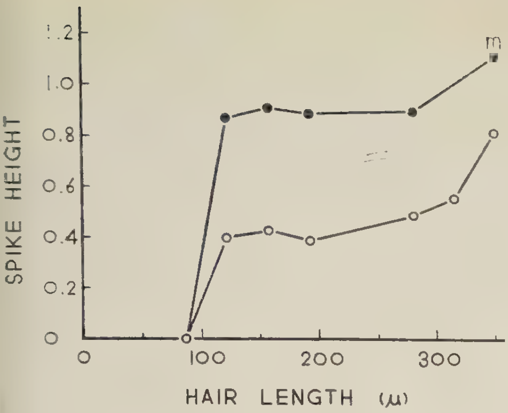

Fig. 2 Relation between the spike height and the length of the hair stump on the labellum from which the spike potentials were recorded, when the distal part of the hair was removed successively. Ordinates, spike heights. Abscissae, lengths of the cut hair on the labellum. The largest hair length represents the intact hair length. Open circles, the heights of spikes which were not modulated by mechanical stimuli. Closed circles, the heights of spikes evoked with mechanical stimulation.

hair. If the distal part of the hair was removed by about 250 μ, both kinds of impulses disappeared completely. From this figure it is safely assumed that both kinds of the spike potentials originated at the proximal part of the hair, although it is in doubt whether the spike potentials occurring with mechanical stimulation originated in the specified mechanoreceptor or in another chemosensory neuron (see discussion).

Spike potentials recorded through side hair wall

The electrical activity of the resting chemosensory hair was recorded by another method. The capillary electrode, which served as the active lead, was brought into contact with the hair wall, and was moved along it until spikes were seen. In some cases, the electrical activity could not be obtained by this method, especially at the end of this season of experiment. Figure 3 shows the results obtained from a hair by this method. Record

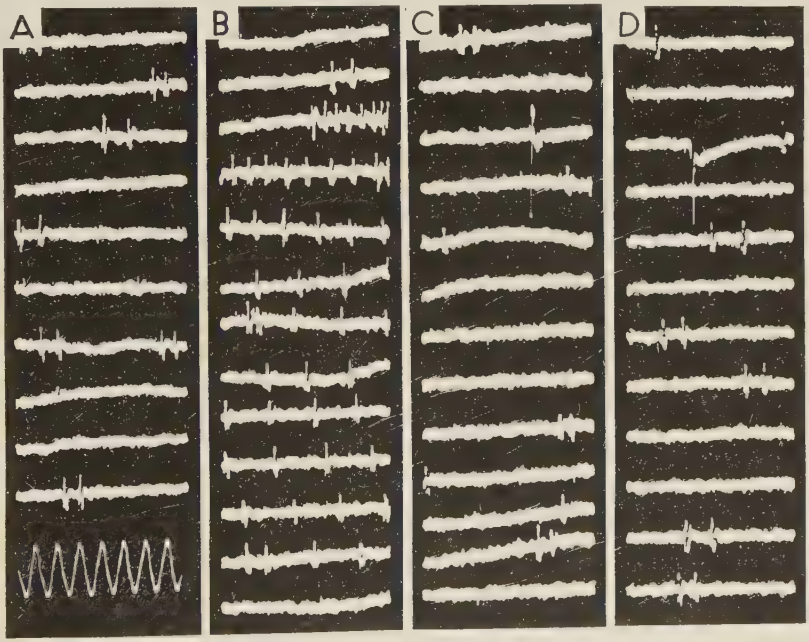

Fig. 3 The responses to chemical stimuli recorded from the intact labellar chemosensory hair through the hair wall. A, impulses from an unstimulated hair. B, response to 1/32 M sucrose solution. C, response to 1/256 M sucrose solution. D, response to ¼ M NaCl solution. Arrows show the time when the solution came in contact with the tip of the hair. Time, 60 cycles/second. Retouched photographs.

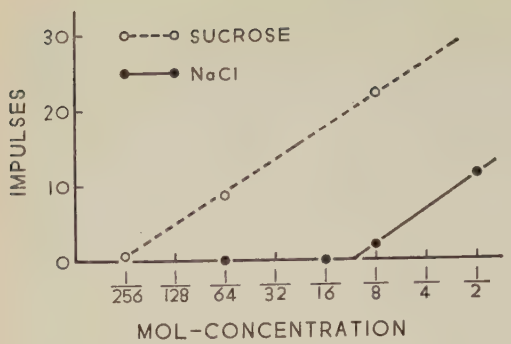

Fig. 4 Relation between the number of impulses and the concentration of the test solution. Ordinates, the number of all impulses occurred in about 0.25 second after the test solution came in contact with the tip of the hair. Abscissae, molar concentrations of the test solutions. Open circles, the responses to sucrose stimulation. Closed circles, the responses to NaCl stimulation. Temperature, 12.5°C.

A in figure 3 shows the electrical activity occurring in a chemosensory hair without chemical stimulation. The tip of the hair was brought into contact with a test solution contained in another capillary, in order to determine whether or not these spike potentials were originated in the chemosensory neuron. In this hair, the frequency of spikes from the resting hair was increased when a sucrose solution of high concentration was brought into contact with the tip of the hair (fig. 3, B). The frequency was sometimes decreased by contact with either dilute sucrose solutions or distilled water (fig. 3 C,), and moreover, this particular hair was not affected by application of NaCl solutions (fig. 3, D). Figure 4 shows the relation between the frequency of spikes and the concentration of the sucrose or NaCl solution brought into contact with the tip of the hair which did respond to NaCl. It is assumed from these results that the chemosensory neuron has spontaneous activity. The results of analysis on the chemoreception by this method will be reported in another paper (Tateda, '59).

DISCUSSION

Recently, impulses generated by chemical stimulation were recorded directly from the inside of the hair after the side wall had been cracked by a micro-capillary (cf., Morita, '59; Tateda, '59). The spikes recorded from the tip were remarkably decreased in height when the inside of the hair was grounded through the cracked wall. However, those recorded through the wall were almost the same in height as those recorded from the hair tip when the inside of the labellum was grounded. From these results it may be assumed that the spike potentials of the chemosensory hair originate at its proximal part. If this is true, then the decrease of the spike height with the length of the hair stump may be explained. If the cut end is sufficiently far from the site of the generation of spikes, the effect of the injury current on the generation site will be slight. If the hair is cut near the site of the spike origin, the spike height will be decreased by the cathodal effect of the injury current; when the cutting point is nearer to the site of origin, the spike will disappear completely. Another possible cause to such decrease in spike height is shown elsewhere (Morita and Yamashita, '59a). On the other hand, one may think that the spike potentials from the cut hair remaining on the labellum generate near the cut end. If such were the case, the spike would be recorded even from the remaining hair which had been cut near the base (cf. fig. 2). Moreover, the decrease in spike height must be attributed to a decrease in the diameter of the neuron. No such change in diameter has been observed.

Wolbarsht and Dethier ('58) reported that the chemosensory neuron was insensitive to mechanical stimulation. If the specified mechanoreceptor sent its distal process to a certain level of the hair, the decrease of the spike height might be explained in the same way as mentioned above. However, if the distal process did not extend beyond the hair base, the decrease in spike height caused by removal of the tip had to be explained in a different way. As pointed out by Wolbarsht and Dethier ('58), it would be possible that a chemosensory neuron "pretended" to respond to mechanical stimulation.

Wolbarsht and Dethier ('58) stated that a cut hair still responded to chemical stimulation, observing S (small) spikes alone on application of D-fructose in contrast to only L (large) spikes in the case of NaCl. However, the spikes of the same size as recorded with the electrode containing NaCl might be recorded as small spikes with the electrode of high resistance which contained non-electrolyte. This might also be the reason why they recorded L spikes of "unchanged" size and M spikes of "increased" size after removal of the hair tip.

It must be considered carefully whether or not the intact chemosensory hair *spontaneously* discharges impulses. Wolbarsht ('58) suggested that the neurons discharged no impulse in the unstimulated condition. An effect of grid current must be considered here. Morita and Takeda ('59) reported that the threshold of a neuron in a tarsal chemosensory hair of *Vanessa* for D.C. stimulation to its tip was in the order of 10^{-10} amperes. It can be assumed, from the results of D.C. stimulation from the cracked side wall of the labellar hair (Morita, '59), that the chemosensory neuron has a higher threshold for D.C. stimulation from the cracked side wall than for that from the tip of the hair. Since the grid current of our pre-amplifier was, at most, in the order of 10^{-12} amperes, it may be assumed that the effect of the grid current on the chemosensory neuron was negligible when the impulses were recorded from the side wall. On the other hand, it is possible, though improbable, that ⅛ M NaCl in the electrode may have a stimulating effect on the side membrane of the chemosensory neuron. Moreover, it is impossible to say that any accidental change in the environment has no effect on the impulse discharge. However, the hyperpolarization induced by some inhibitors at the receptor region (Morita and Yamashita, '59b) indicates that the receptor membrane is somewhat depolarized in the resting state. It is most probable that this depolarization initiates the "spontaneous" impulses of low frequency.

As stated by Hodgson and Roeder ('56), it was impossible to record the resting activity when the recording electrode was used simultaneously as the chemical stimulator. Moreover, with this method, it was impossible, unless some difficulties were overcome, to record the response to a pure solution of non-electrolyte. As mentioned above, we could record this response by keeping the active electrode, which was independent of the stimulating solution, in contact with the side wall of the sensory hair. It seems unlikely this method recorded spikes from the tip, since their heights were not affected by application of non-electrolytes to the tip.

SUMMARY

The investigation was undertaken to localize the generation site of the spike potentials recorded from the labellar chemosensory hairs of the fly, *Lucilia caeser*.

The spike potentials were never recorded from the isolated distal part of the hair whose tip was kept intact. On the other hand, they could be recorded from the chemosensory hair, whose distal part was removed, although their frequency was not changed during the whole course of chemical stimulation.

The electrical activity of the intact hair was recorded by the active electrode through the side wall of the hair.

From these results it was concluded that the spike potentials were generated at the proximal part of the hair, although the chemoreceptor was assumed to be on the tip of the hair.

ACKNOWLEDGMENT

The authors wish to express their thanks to Professor M. Kuwabara of Kyushu University for his kind suggestions and criticisms.

LITERATURE CITED

Dethier, V. G. 1956 Chemoreceptor mechanism. Molecular structure and functional activity of nerve cells. Publ. No. 1, Amer. Inst. Biol. Sci., Washington, D. C.

Hodgson, E. S. 1958 Electrophysiological studies of arthropod chemoreception. III. Chemoreceptors of terrestrial and freshwater arthropods. Biol. Bull., *115:* 114–125.

Hodgson, E. S., and K. D. Roeder 1956 Electrophysiological studies of arthropod chemoreception. I. General properties of the labellar chemoreceptors of Diptera. J. Cell. and Comp. Physiol., *48:* 51–76.

Morita, H. 1959 Initiation of spike potentials in contact chemosensory hairs of insects. III. D. C. stimulation and generator potential of labellar chemoreceptor of *Calliphora.* Ibid., *54:* 189.

Morita, H., S. Doira, K. Takeda and M. Kuwabara 1957 Electrical response of contact chemoreceptor on tarsus of the butterfly, *Vanessa indica.* Mem. Fac. Sci. Kyushu Univ., Ser. E (Biol.), *2:* 119–139.

Morita, H., and K. Takeda 1959 Initiation of spike potentials in contact chemosensory hairs of insects. II. The effect of electric current on tarsal chemosensory hairs of *Vanessa*. J. Cell. and Comp. Physiol., *54:* 177.

Morita, H., and S. Yamashita 1959a The backfiring of impulses in a labellar chemosensory hair of the fly. Mem. Fac. Sci. Kyushu Univ., Ser. E (biol.), *3:* 81–87.

——— 1959b The generator potential of insect chemoreceptor. *130:* 922

Tateda, H. 1959 The electrical responses of the labellar chemosensory hairs of the fly to sucrose, sodium chloride, quinine hydrochloride and acetic acid. In preparation.

Wolbarsht, M. L., and V. G. Dethier 1958 Electrical activity in the chemoreceptors of the blowfly. I. Responses to chemical and mechanical stimulation. J. Gen. Physiol., *42:* 393–412.

Wolbarsht, M. L. 1958 Electrical activity in the chemoreceptors of the blowfly. II. Responses to electrical stimulation. Ibid., *42:* 413–428.

Initiation of Spike Potentials in Contact Chemosensory Hairs of Insects

II. THE EFFECT OF ELECTRIC CURRENT ON TARSAL CHEMOSENSORY HAIRS OF VANESSA[1]

HIROMICHI MORITA AND KIMIHISA TAKEDA
Department of Biology, Faculty of Science, Kyushu University,
Fukuoka, Japan

Taste organs of vertebrates have been studied for many years by recording the afferent impulses (Pumphrey, '35; Zotterman, '35), while it has just recently been possible to study the contact chemoreceptors of invertebrates electrophysiologically (Hodgson et al., '55; Barber, '56; Morita et al., '57; Stürckow and Quadbeck, '58). Nevertheless, as Hodgson and Roeder ('56) pointed out, the recording of the electrical response of the primary chemosensory neuron itself seems to have been successful only in chemosensory hairs of insects. Thus, they reveal themselves as the most advantageous material for the fundamental study on the chemoreception. From this point of view, the effect of the electrical current on the chemosensory hair of *Vanessa* tarsus was studied in the present work. The general rule of the electrical effect was obtained on the chemoreceptive responses to both electrolyte and non-electrolyte as well as on its response to the mechanical stimulation.

MATERIAL AND METHODS

The larvae of the butterfly, *Vanessa indica,* were collected and reared in the laboratory. The experiments were performed in the winter or early in the summer with the tarsal chemosensory hair of the butterfly. The recording methods of the chemosensory response were the same as those described in the previous paper (Morita et al., '57). In the present study, however, a bridge circuit was built as shown in figure 1a. This was necessary because an electrical pulse of large amplitude had to be added during the application of a stimulating solution. But in other cases, the circuit of figure 1b was used. The calibration pulse and the stimulating potential were generated with v_c and v_s, respectively, in these figures. Their polarities are designated as plus when they have the same polarity as described in these figures, and *vice versa.* The term "anelectrotonus" or "anodal" polarization is used in this paper when the electrical current flows from the test solution to the inside of the tip of the chemosensory hair; and "catelectrotonus" or "cathodal" polarization in the case of the reversed direction.

The composition of the solution used is indicated as the final concentrations of component solutes in terms of molarities. The solution indicated as M/2 NaCl + M/4 sucrose, for example, contained ½ mole NaCl and ¼ mole sucrose per liter solution.

RESULTS

Strength-duration curve

A *Vanessa* tarsal chemosensory hair was exposed to a solution of M/8 NaCl for such a long time that the hair no longer discharged a spike potential because of a kind of fatigue. After the spikes were abolished, an anodal rectangular pulse (about 1/15 sec.) was added. The bridge circuit was not designed to balance the capacitance factor; therefore, the distortion of the base-line due to the pulse remained clearly visible. This remaining distortion only was seen when the weak pulse was added (fig. 2a), while a spike poten-

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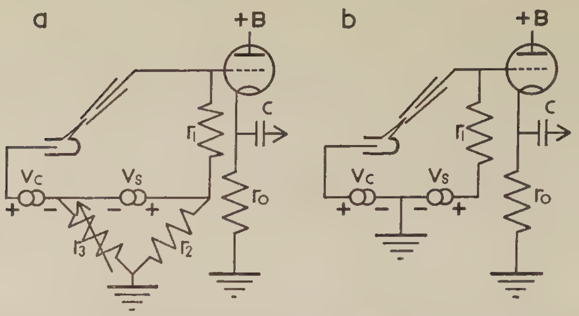

Fig. 1 The schematic representation of the recording and stimulation systems. *a*, the arrangement used for the electrical stimulation starting during the chemical stimulation; *b*, in the case of the two kinds of stimulations starting at the same time. *c*, 0.005 μF; r_o, 200 kΩ; r_1, 50 MΩ; r_2, 5 kΩ; r_3, variable resistance of 5 kΩ; V_c, source of calibration; V_s, source of stimulation pulse or electrotonus.

tial occurred when the pulse was a little stronger (fig. 2b). The electrical polarity of the spike, of course, coincided with that observed in the previous works on the tarsal chemosensory hair of *Vanessa* (Morita et al., '57) and on the *Lucilia* labellar hair (Tateda and Morita, '59). With the stronger pulse, the spike potential occurred the sooner after the initiation of the rectangular pulse, and the number of spikes occurring in each period of the pulse increased (fig. 2, b to o). Such spikes were not evoked with the cathodal pulse of the same amplitude as the strongest one in the anodal series (fig. 2p). In these records, two types of spikes (one small and the other large) are seen. In some cases (fig. 2: b, c, g, etc.), the first spike of each type seems to be superimposed on the other and it is difficult to detect the small spike. Hence, in figure 3, the strength of the pulse was plotted against the latent time of the first spike of the large type only. This curve is comparable with the intensity-duration curve well established on other irritable tissues or cells. The *it-t* relation also is shown to be in the same case, where *i* and *t* represent the strength of stimulus and the latent time, respectively.

Responses under electrotonus

In the experiment shown in figures 2 and 3, the rectangular pulse was added while the chemosensory hair was in contact with the solution. On the other hand, the solution could be applied to the hair after the potential difference had been established between the solution and the hair. In this case, the circuit b shown in figure 1 was used. The e.m.f. was generated with v_s, and then the stimulating

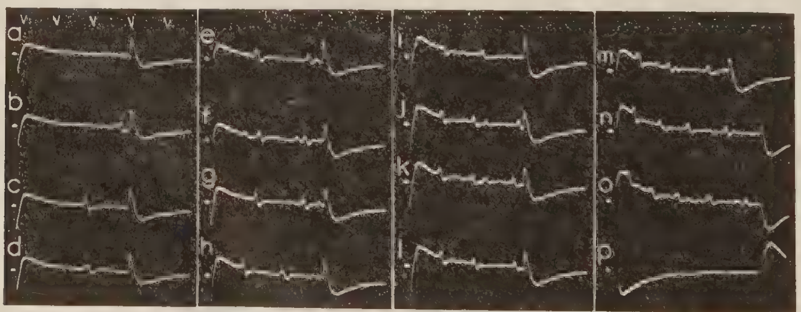

Fig. 2 The response of the chemosensory hair of *Vanessa* tarsus to the electrical pulse during the adaptation of the hair to M/8 NaCl. The *anodal* pulses were made stronger from *a* to *o*. The pulse, the same as that of *o* in strength but reversed in polarity, was added in *p*. Time scale, 1/60 sec. 13°C.

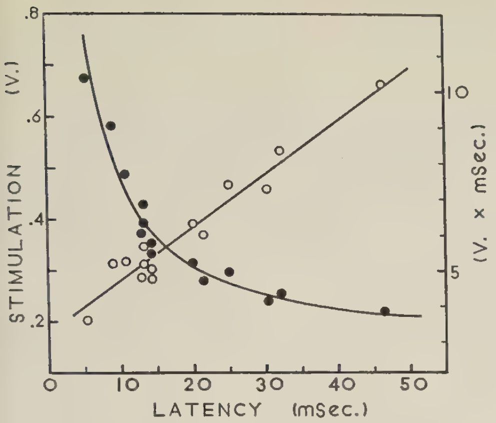

Fig. 3 The relation between the strength of electrical stimulation and the time from the start of the electrical pulse to the initiation of the first spike potential. The *it-t* relation also is shown. Plotted from the records shown in fig. 2.

solution was administered to the hair tip for a few seconds, near the end of the administration the calibration pulse being added through v_c. Thus, the spike potentials could be recorded under the various strength of e.m.f. from the same hair with the same solution. One of the results of such experiments is shown in figure 4. In this experiment, a M/32 NaCl solution was applied to the hair tip. The frequency distributions of the spikes during the first one of the repeated sweeps (about 1.2 second) are plotted against their heights which were measured by mm-unit on the enlarged photographs and transferred on a logarithmic scale. The calibration pulse of +2 mv D.C. induced the brief monophasic deflection of the beam—this was due to the interposition of the coupling condenser of small capacitance—and its base-line was recorded to be wide because of the fairly slow sweep and high noises. The height of deflection due to the calibration pulse, therefore, was estimated with a relatively large inaccuracy which corresponded to the width of the base-line and was shown by the shaded area in figure 4. The spike potentials occurring in sensory elements were diphasic at any rate, and were recorded as deflections above noise level on both sides of the base-line. Therefore, their amplitudes were estimated accurately. The e.m.f. under which the histograms of a to h in figure 4 were obtained were +800, +400, +200, +100, 0, −100, −200, −400 mv, respectively. In this figure, the estimated value of deflection height due to the calibration pulse fluc-

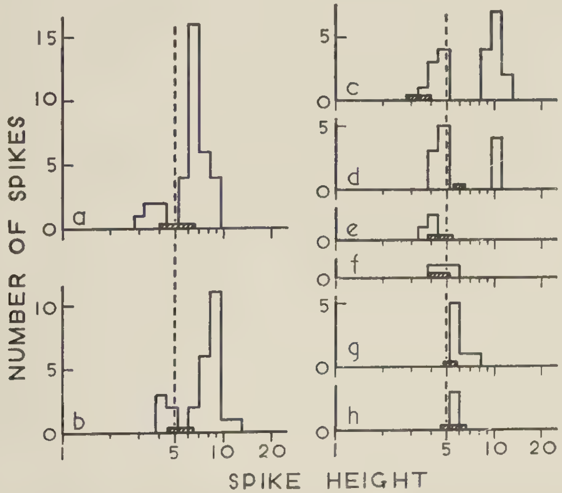

Fig. 4 The distributions of the spike height, which was measured my mm-unit on the enlarged photographs. The tarsal chemosensory hair of *Vanessa* was exposed to M/32 NaCl under various strength of electrotonus. The strengths of electrotonus in *a* to *h* were +0.8, +0.4, +0.2, +0.1, 0, −0.1, −0.2, −0.4, volts, respectively. The shaded area shows the deflection height of the calibration pulse of +2 mv. The broken line indicates the height around which the calibration pulse fluctuates. The spikes were measured during the first one of the repeated sweeps, about 1.2 sec. 11°C .

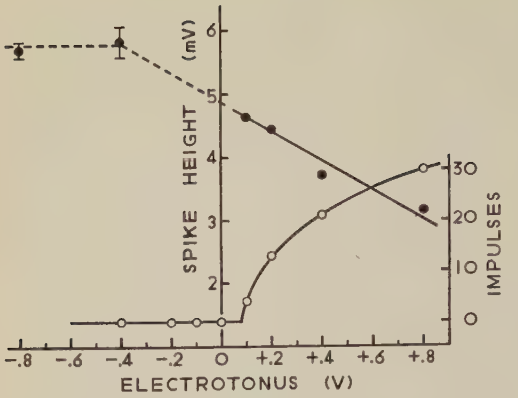

Fig. 5 The height and the frequency of spikes against the strength of electrotonus under which the spikes were recorded. Plotted from the data of spikes of large type shown in fig. 4. Closed and open circles indicate the height and frequency of spikes, respectively.

tuates around a vertical broken line crossing abscissae at the points of 5 mm. (In fig. 5, therefore, spike height is plotted in mv, assuming that 5 mm of deflection corresponds to 2 mv.) Each histogram contains two groups of spikes (fig. 4: a to d), and it is safe to assume that they originated in two different sensory elements respectively. On the other hand, the groups of spikes in figure 4: e, f, g and h is thought to have occurred in the same sensory element in which the spikes of small type in figure 4: a to d occurred: It is because a group of spikes as large as the spikes of large type in figure 4d often occurred after the e.m.f. was removed. The spikes of large type had certain definite tendencies; that is, they increased in amplitude and decreased in number with decrease of strength of the e.m.f. These tendencies are shown more clearly in figure 5. In this figure, the mean value of spike height and the frequency of spikes are plotted against the strength of the added e.m.f. This figure shows that the number of spikes increases exponentially with the increase of anelectrotonus. At the same time, it can be said that the spike height within a certain limit is proportional to the strength of the electrotonus. This proportionality is seen in other results of such experiments on *Vanessa*, and also on labellar chemosensory hairs of *Calliphora* (Morita, '59). In this experiment, the heights of spikes occurring after cessation of the electrotonus of −0.4 and −0.8 v were measured, and are plotted hypothetically on the positions corresponding to −0.4 and −0.8 v in this figure. This procedure might be thought to conflict with the fact that no electrotonus was applied to the hair at those times. However, in general, the cell membrane takes a certain period to return to its resting level after removal of an electrotonus; and the discharge of spikes in the chemosensory hair continued only for a brief period (less than 0.2 second) after the catelectrotonus was taken off. Therefore, the plotting of heights as shown in this figure is considered to be fairly reasonable. These regular relations of the spike height and the frequency of spikes with regard to the added e.m.f. are not seen in the case of the spikes of small type in figure 4. In this case, the above-mentioned tendencies might be masked by the fact that such small spikes could not be clearly distinguished from the noises (as in the same case, see fig. 6A). In fact, these spikes showed the same tendencies as the large-typed spikes when M/8 NaCl was administered to the same sensory hair and the height of each spike was larger than in the case of M/32 NaCl.

Sucrose-receptor under influences of electrotonus

From the results mentioned above, it might be thought that the electrotonic effect would act through the electrophoretic application of a cation (in these cases, Na$^+$) on the chemoreceptor sensitive to the cation. This is not an adequate explanation of the result of an experiment in which the chemoreceptive response is concerned with non-electrolyte. Figure 6 shows the result of such an experiment, which was exactly the same in procedure as that of figure 4. The histograms in figure 7, also, were obtained in the same manner from the records shown in figure 6. In this case, however, the spike heights are represented in mv, assuming that the deflection height of the calibration pulse measured from the median point of the base-line corresponds to 2 mv in each histogram. The heights thus assumed to correspond to 2 mv in histograms a to f were

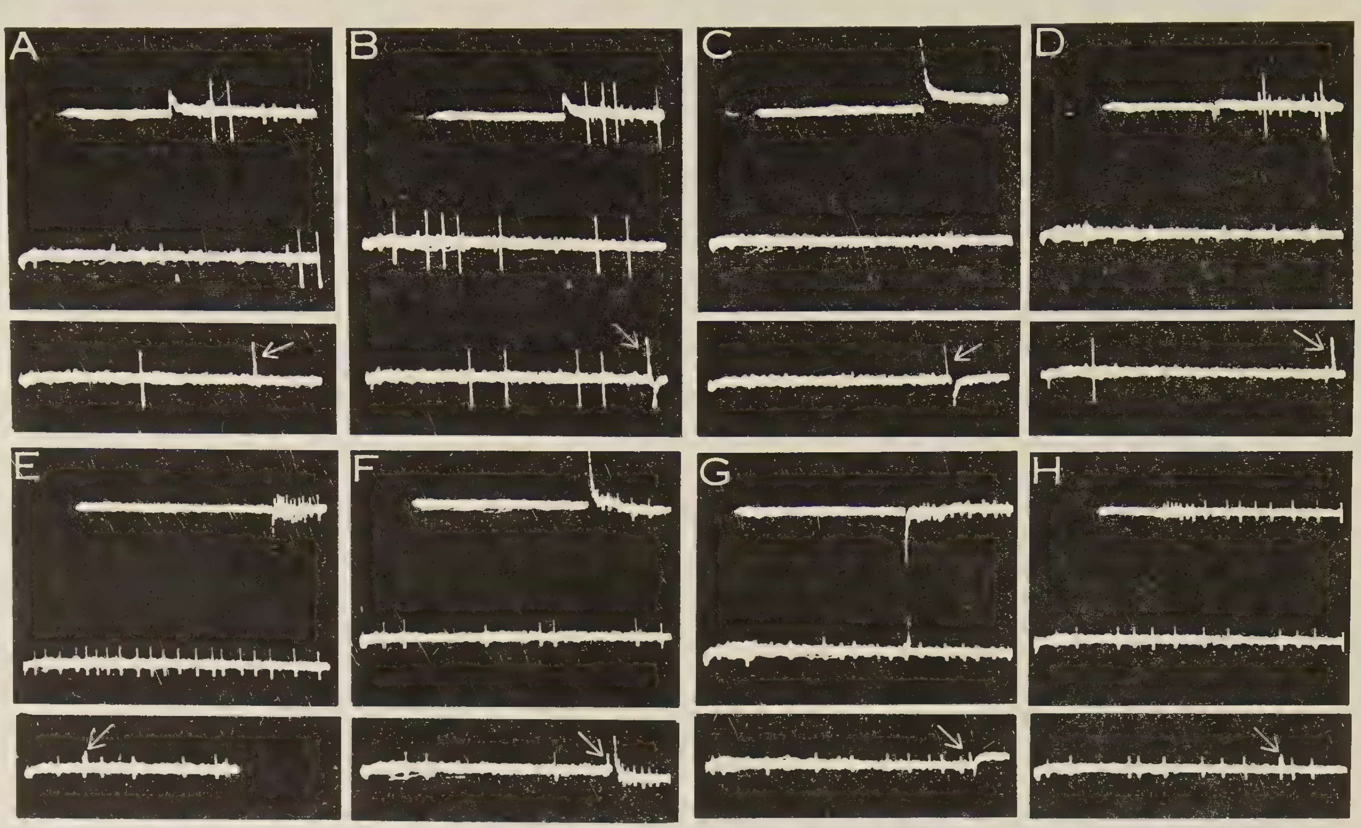

Fig. 6 The effect of the electrotonus on the spikes initiated by sucrose. The first and the last ones of the recorded sweeps are shown; the white band within each record indicates the omission of a few, recorded sweeps. Arrows indicate the calibration pulse of +2 mv. One sweep corresponds to about 1.2 sec. Some spikes were retouched. The test solution and the electrotonus added was: M/32 NaCl, 0 mv in *A*; M/32 NaCl, +50 mv in *B*; M/32 NaCl + M/20 $CaCl_2$, +200 mv in *C*; M/32 NaCl, 0 mv in *D*; M/2 sucrose + M/32 NaCl, 0 mv in *E*; M/2 sucrose + M/32 NaCl, −400 mv in *F*; M/2 sucrose + M/32 NaCl, +400 mv in *G*; M/2 sucrose + M/32 NaCl, 0 mv in *H*. The records were obtained in this order. 10°C.

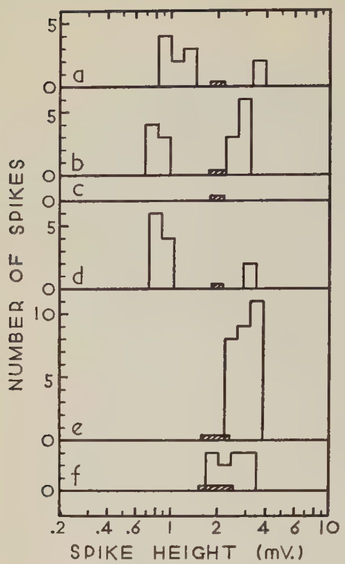

Fig. 7 Histograms obtained from the records of fig. 6. *a*, *b*, *c*, etc. in this figure correspond to *A*, *B*, *C*, etc. in fig. 6, respectively, except for *G* and *H*.

8.1, 9.7, 6.8, 8.8, 3.8 and 3.9 mm, respectively, and the widths of base-line are indicated as shaded areas.

The trains of spikes in records E, F, G, and H in figure 6 are considered naturally to have occurred in one and the same sensory element. Furthermore, these spikes are safely judged to represent a response to sucrose, because the only one difference between the conditions in A and E is related to sucrose. Now, from the illustration of figure 7, it is concluded that the action potentials of large type occurring under applications of M/32 NaCl (fig. 6: A, B and D) have approximately the same amplitude as those responding to sucrose (fig. 6: E to H). It can not be concluded, of course, that these spikes representing the response to sucrose were initiated in the same sensory element that discharged the large spikes under application of M/32 NaCl. Both of them, however, behaved similarly in response to the electrotonus. It is noteworthy that the spikes responding to the non-electrolyte are controlled by an application of electrotonus in the same way as those initiated by pure NaCl solutions. It should also be noticed that no spike was elicited with the existence of M/20 $CaCl_2$ in a M/32 NaCl solution even under the anelectrotonus as strong as 200 mv (fig. 6C). This failure of response was not due to a fatigue, because this chemosensory hair responded to M/32 NaCl immediately afterwards (fig. 6D).

Effect of NaCl on sucrose-receptor

Studying the partial adaptation of labellar chemosensory hairs of flies, Tateda ('59) obtained interesting results. That is: (1) Immediately after the continuous stimulation (two minutes) by sucrose, the response to sucrose was blocked selectively. (2) On the other hand, after the same kind of stimulation by NaCl, both responses to sucrose and to NaCl were depressed deeply for some period. These results which look like an adaptation to NaCl might present an idea that the sucrose-receptor in the chemosensory hair of insects is stimulated by Na. If such was the case, the fact that the sucrose-receptor is controlled by the electrical current in the same way as a sodium-receptor would be explained merely as the effect of the electrophoretic application of sodium to the sucrose-receptor. On the other hand, Hodgson ('57) reported, on the labellar chemosensory hair of

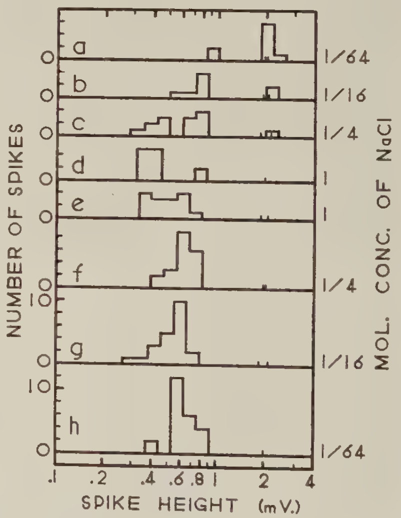

Fig. 8 Inhibitory effect of NaCl upon sucrose-receptor. Pure NaCl solution of indicated concentration was applied in *a* to *d*; M/2 sucrose solution containing NaCl of indicated concentration was applied in *e* to *h*. Spikes were measured for the first 0.2 sec. of stimulation. Marks around a point of 2 mv on abscissae represent calibration spikes. 26–26.5°C.

Phormia, that stimulation of the *L* fiber is associated with a decrease in the frequency of *S* spikes.

To clarify the effect of NaCl on the sucrose-receptor of *Vanessa* tarsus, a result of experiments is presented in figure 8. This series of experiments was run on 5 different hairs, located on two different butterflies. All results showed, more or less, the same tendency as shown in figure 8. The histograms in this figure were obtained, as usual, on one chemosensory hair for the first 0.2 second of stimulation, but the experiment differed in procedure as follows: (1) The chemosensory impulses were picked up with the aid of the head stage of amplifier without a grid leak. Thus, the electrical influence of the potential difference between the recording electrodes was eliminated. (2) The impulses were tape-recorded together with a rectangular calibration pulse. Because of the characteristic of the tape recorder, this rectangular pulse was recorded as two mutually mirror-imaged spikes corresponding to start and end of the pulse. Therefore, the mean of the heights of the two spikes was taken at 2 mv in each of these histograms, the appropriate positions of the two spikes also being indicated. Histograms a to d show the results of applications of the pure NaCl solutions of the indicated concentrations; while histograms e to h represent those of applications of the M/2 sucrose solution containing NaCl of the indicated concentrations. It is clear, from this illustration, that the group of impulses whose heights distribute around 0.6 mv represent the response to sucrose. It is also evident that the response to sucrose was deeply depressed by 1 M NaCl, and slightly depressed by M/4 NaCl. As the interval between tests was sufficiently long (more than 10 minutes) and the test was carried out in the order of a to h, the results were completely free from the effect of adaptation.

Thus, concerning the results shown in figures 6 and 7, it should be concluded that a sucrose-receptor in the tarsal chemosensory hair of *Vanessa* is not stimulated by Na^+ carried electrophoretically, but is controlled by an electrical current itself. Otherwise, Cl^- applied to the receptor must be considered to depress the response of the receptor. This is difficult to suppose, because it is known that an anion is inert in almost all cases of chemoreception.

The result of figure 8 indicates some other interesting characters of the chemosensory hair, although apart from the primary interest of this paper. It seems that there were at least 4 chemosensory neurons in this chemosensory hair: The first discharged impulses of approximately 2 mv and responded to NaCl of concentration below M/4. Impulses of the second were about 0.8 mv and responding to NaCl in a range of concentration 1 M to M/64. The third responded to NaCl of concentration above M/4 with impulses of 0.4–0.5 mv. Some of them seem to have optimal concentration to respond. The last is the sucrose-receptor mentioned just above. A precise biological significance, however, is not clear at present, concerning such a differentiation between the chemosensory neurons sensitive to NaCl.

Impulses responding to mechanical stimulation

The response of the chemosensory neuron to the mechanical stimulation such as bending or vibration has been reported by Hodgson and Roeder ('56) on the labellar chemosensory hair of the fly. On the other hand, Wolbarsht and Dethier ('58) postulated that the spikes evoked by mechanical stimuli were generated only in specialized mechanoreceptors. As reported in the blowfly, in *Vanessa* also, the spike potentials responding to the mechanical stimulus have such a characteristic shape that they can be easily distinguished from the spikes representing the response to chemical stimulations. Even with a RC-amplifier of small time constant, this difference in shape can be clearly seen. Such an example is shown in figure 9A. The train of spikes of long time course is seen when the hair is bent with the capillary electrode covering its tip. It has an initial phase of facilitation (increase in spike height and in frequency) and a falling phase of adaptation (decrease in spike height and in frequency). The characteristic difference in shape between the spikes which are evoked with mechanical and chemical stimuli is seen much more clearly in figure 9B. In this case, the spikes were recorded on the

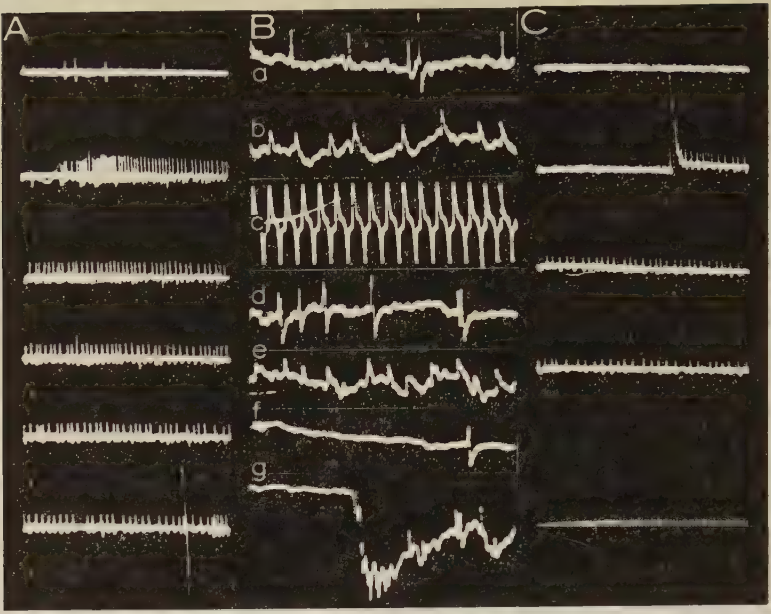

Fig. 9 The response to mechanical stimulation and electrotonus. *A*: The typical response of the hair to bending. The hair was bent on the way at the second sweep. The calibration pulse of brief rectangular pulse, +2 mv is diphasically seen at the last sweep. Time scale, the same as that in *C*. 12.5°C. *B*: The records to show clearly the difference in shape, between the spikes responding to chemical stimulation and those responding to mechanical stimulation. The time constant of the amplifier was about ½ sec. Records *a* and *b* were obtained from one hair, and records *d* to *g* from another. *a*, M/8 NaCl, without bending the hair. *b*, M/8 NaCl, with bending. *c*, 1/60 sec.; this scale applies to all records in *B*. *d*, M/8 NaCl, without bending. *e*, M/8 NaCl, with bending. *f*, after the tip of the hair was cut off, M/8 NaCl, without bending. *g*, the same as above, M/8 NaCl, with bending. 13°C. *C*: The effect of electrotonus upon the response to the mechanical stimulation. The spikes are not seen on bending the hair under the electrotonus of —400 mv, but they appear after cessation of the electrotonus. The calibration pulse, +2 mv, are seen immediately before the cessation of the electrotonus. Time, 1/60 sec. and 1/10 sec. 11°C.

faster sweep, and the RC-amplifier used had a time constant of 400 times as large as the one used in other experiments. The typical shapes of the spikes which represent the responses to chemical and mechanical stimuli are respectively shown in figure 9B: a and b. From these records, it can be seen that the spikes responding to the mechanical stimulus have a much longer time course than those responding to the chemical stimulus. The spikes can be recorded even from the cut end of the hair without bending (fig. 9Bf) as well as in the case of bending (fig. 9Bg, the same hair with that in fig. 9B: d and e). It should be noted that the base-line is deflected slowly to the negative side at the moment of bending, being superimposed by spike potentials. This slow deflection to the negative side is commonly observed also in the intact hair (in many species of flies as well as in *Vanessa*) in the case of mechanical stimulation. It is supposed that this potential is concerned with the initiation of the spikes.

Now, under the effect of the electrical current made to flow, such spikes responding to mechanical stimulation behave in the same way as the spikes responding to chemical stimulation. In the experiment in figure 9C, a chemosensory hair of *Vanessa* tarsus was bent after application of a test solution kept previously at —400 mv. No spike was seen under this condition, but a train of spikes occurred as soon as the electrotonus was taken off.

Those spike potentials elicited by bending the hair seem to be related with the chemoreception. This is considered not only from the same mode of their response to the electrical polarization as that of the chemosensory impulses, but also from their "adaptation to chemical stimulation": The recording electrode containing M/32 NaCl was brought in contact with the tip of a chemosensory hair, and it was ascertained that this hair responded to bending. The spikes, however, were not initiated by bending the hair after its tip had been kept in the M/32 NaCl for one minute. On the other hand, the spikes did occur by bending after the hair tip had been kept free in the air for one minute. This was quite reproducible for several repetitions. According to Wolbarsht and Dethier ('58), chemosensory neurons in a labellar hair of the blow-fly discharge spikes when the hair is bent, only because a concentration change in the solution around the tip is produced. In the present study, no special care was exercised to prevent this change. Therefore, it is possible that the spikes mentioned just above may have been initiated in chemosensory neurons, not by mechanical stimulation, but by the concentration change in the solution. In fact, as indicated elsewehre (Morita, '59), spikes in chemosensory neurons may be modified in shape by bending.

DISCUSSION

D.C. stimulation on chemoreceptors of other animals

The effect of electrical current was observed in the mammalian chemoreceptor by Pfaffmann ('41, '55). He reported that steady currents with anode on the tongue elicited the typical asynchronous nerve activity and that cathodal polarization of the tongue reduced, or even stopped completely, any resting activity. His observation is completely consistent with the present results, but the precise arrangement of electrodes which made the currents flow was not indicated in his paper. Therefore, the consistency is questionable to be comparable to what extent.

On the chemosensory hair of the labellum of the blow-fly, Dethier and Wolbarsht ('56) reported from a behavioral study that stimulation by cathodal current was possible at both the intact tip and cut end of the hair. However, the results of the electrophysiological studies on labellar chemosensory hairs of flies (Wolbarsht, '58; Morita, '59) well coincide with those described here. Wolbarsht ('58) interpreted this superficial conflict between the results of the behavioral and electrophysiological studies.

The locus where electrical current is effective

From figure 3, the threshold electrical current that corresponds to rheobase can be computated approximately as 2×10^{-9} amperes. There, the resistance of the sensory hair tip was assumed to be 70–80 megohms (Morita and Takeda, '57). The tarsal chemosensory hair of *Vanessa* contains several processes from the sensory cells. Accordingly, the magnitude of threshold electrical current which initiates a spike in one of the neurons should fall in the order of 10^{-10} amperes. The result of this computation indicates the surprisingly high efficiency of electrical stimulation, as the magnitude of the threshold electrical current, even in the most excitable structure, i.e., a single node of a myelineated nerve fiber, is in a range of $3\text{--}6 \times 10^{-10}$ ampere. Such high efficiency is inconsistent with any assumption that the nerve or something else in *Vanessa* tarsus would respond to the electrical current somewhere near the cathodal electrode. This is also denied by the result of simultaneous recording from two adjacent chemosensory hairs on one and the same tarsus: One of them applied with anelectrotonus showed the acceleration of the occurrence of spikes and the other without electrotonus showed no such acceleration.

Even for the last few years there are many works dealing with D.C. stimulation on various sensory organs (e.g., Katsuki, Yoshino and Chen, '51; Edward, '55; Murray, '56). Concerning the relation between the strength of the applied current and the impulse frequency, some of these results indicate the direct proportionality between them and others show the same between the frequency and the logarithm of the strength. However, all the authors agreed in the opinion that the current initiated the impulses on the side of nerve fibers. The case of chemoreceptors is not so simple as that of other receptors, because it is possible that the chemoreceptor surface is stimulated by ions carried electrophoretically. However, the *i-t* and the *it-t* relations shown in figure 3 suggest strongly that spikes are elicited directly by electrical stimulation at the region of the chemosensory neuron where spikes occur in an all-or-none manner. The fact that the impulse frequency is related exponentially to the strength of electrotonus as shown in figure 5 is not characteristic of receptors only. In fact, on some single fiber preparation of nerve, the proportionality between the interval of impulses and the logarithm of the strength of stimulus voltage has been recognized (Tasaki, '52). From the tendency shown in the same figure that the spike height is proportional to the strength of the electrotonus, it is reasonable to assume that the electrical current stimulates the neuron at the generation site of the spikes. Last and decisively, this assumption is supported by the fact that a sucrose-receptor which is to be inhibited by NaCl is affected in the same way as Na-receptor by electrotonus applied to the hair tip (figs. 6–8). As to the inhibitory effect of calcium as shown in figure 6, two kinds of explanations are possible: (1) The electrical conductivity of the chemoreceptor surface may be made reduced by calcium, and almost all parts of the current may flow through the interstitial space. (2) Calcium may hyperpolarize the receptor surface, and the cathodal effect of anelectrotonus applied to the hair tip may be antagonized at the generation site of spikes. The assumption illustrated above, of course, does not imply that any kind of receptor, such as Na-receptor, will not be affected at all at the receptor region by electrical current. It is quite evident that the generation site of spikes elicited by electrical stimulations, which was discussed just above, coincides with the generation site of spikes initiated by chemical stimulations, which was localized on the proximal side of the chemosensory hair (Tateda and Morita, '59).

Criticism of the results in the previous study

In the previous work (Morita et al., '57), a head stage of amplifier had a grid leak, whose resistance was as high as 50 megohms. Under such circumstances, the chemosensory neurons might be exposed to the electrical polarization which must be built up between the two recording electrodes. As shown in figure 6F, though its frequency is lowered to a fairly large extent, the train of spikes representing the response to sucrose is seen to be even with the cathodal polarization as large as 400 mv. Therefore, most of the conclusions made in the preceding paper are considered to still stand. The failure of response to acetic acid, however, was to be questioned strongly; on every application of acetic acid, the large distortion of the base-line was detected, which suggested the great difference of potential between the electrodes. For this reason, the response to acetic acid was reinvestigated on *Vanessa* tarsal chemosensory hairs using an amplifier without a grid leak. In this way, the train of spikes could be recorded when 1 M acetic acid was applied, but not when M/10 acetic acid was applied. The sensory elements responding specifically to acid needs to be studied more extensively.

SUMMARY

1. The electrical pulse was applied to the tip of the tarsal chemosensory hair of *Vanessa* with the capillary electrode containing a NaCl solution, and the intensity-duration curve was obtained. This curve was quite similar in shape to those obtained in other irritable tissues or cells, but the spikes occurred when the sufficiently strong *anodal* pulse was added to the tip of the hair.

2. The chemosensory impulses were recorded under various strengths of electrotonus, and their height and frequency were measured. The frequency was increased exponentially but the height was decreased linearly, with the increase of anelectrotonus.

3. The electrotonus applied to the hair tip was in a same way of effect on the occurrence of spikes representing the responses to NaCl, to sucrose and to mechanical stimulations. It was shown, on the other hand, that NaCl of high concentration inhibited the response to sucrose.

4. From these results, it should be concluded that the applied electrical current

controls the occurrence of spikes at their generation site which is located on the proximal side of the chemosensory hair.

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LITERATURE CITED

Barber, S. B. 1956 Chemoreception and proprioception in *Limulus*. J. Exp. Zool., *131:* 51–69.

Dethier, V. G. and M. L. Wolbarsht 1956 The electron microscopy of chemosensory hairs. Experientia, *8:* 335–337.

Edwards, C. 1955 Changes in the discharge from a muscle spindle produced by electrotonus in the sensory nerve. J. Physiol., *127:* 636–640.

Hodgson, E. S. 1957 Electrophysiological studies on arthropod chemoreception. II. Responses of labellar chemoreceptors of the blowfly to stimulation by carbohydrates. J. ins. Physiol., *1:* 240–247.

Hodgson, E. S., J. Y. Lettvin and K. D. Roeder 1955 Physiology of a primary chemoreceptor unit. Science, *122:* 417–418.

Hodgson, E. S. and K. D. Roeder 1956 Electrophysiological studies of arthropod chemoreception. I. General properties of the labellar chemoreceptors of diptera. J. Cell. and Comp. Physiol., *48:* 51–75.

Katsuki, Y., S. Yoshino and J. Chen 1951 Action current of single lateral-line nerve fiber of fish. II. On the discharge due to stimulation. Jap. J. Physiol., *1:* 179–194.

Morita, H. 1959 Initiation of spike potentials in contact chemosensory hairs of insects. III. D.C. stimulation and generator potential of labellar chemoreceptor of *Calliphora*. J. Cell. and Comp. Physiol., *54:* 189.

Morita, H., S. Doira, K. Takeda and M. Kuwabara 1957 Electrical response of contact chemoreceptor on tarsus of the butterfly, *Vanessa indica*. Mem. Fac. Sci. Kyushu Univ. Japan, Ser. E (Biol.), *2:* 119–139.

Morita, H., and K. Takeda 1957 The electrical resistance of the tarsal chemosensory hair of the butterfly, *Vanessa indica*. Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool., *13:* 465–469.

Murray, R. W. 1956 The response of the lateralis organs of *Xenopus laevis* to electrical stimulation by direct current. J. Physiol., *134:* 408–420.

Pfaffmann, C. 1941 Gustatory afferent impulses. J. Cell. and Comp. Physiol., *17:* 243–258.

——— 1955 Gustatory nerve impulses in rat, cat and rabbit. J. Neurophysiol., *18:* 429–440.

Pumphrey, R. J. 1935 Nerve impulses from receptors in the mouth of the frog. J. Cell. and Comp. Physiol., *6:* 457–467.

Stürckow, B., and G. Quadbeck 1958 Elektrophysiologische Untersuchungen über den Geschmakcssinn des Kartoffelkäfers, *Leptinotarsa decemlineata* Say. Z. Naturforschg., *13b:* 93–95.

Tasaki, T. 1952 Cold Spring Harbor Symposium, XVII, p. 94.

Tateda, H. 1959 The electrical responses of the labellar chemosensory hairs of the fly to sucrose, sodium chloride, quinine hydrochloride, and acetic acid. In preparation.

Tateda, H., and H. Morita 1959 Initiation of spike potentials in contact chemosensory hairs of insects. I. Generation site of the recorded spike potentials. J. Cell. and Comp. Physiol., *54:* 171.

Wolbarsht, M. L. 1958 Electrical activity in the chemoreceptors of the blowfly. II. Responses to electrical stimulation. J. Gen. Physiol., *42:* 413–428.

Wolbarsht, M. L., and V. G. Dethier 1958 Electrical activity in the chemoreceptors of the blowfly. I. Responses to chemical and mechanical stimulation. J. Gen. Physiol., *42:* 393–412.

Zotterman, Y. 1935 Action potentials in the glossopharyngeal nerve and in the chorda tympani. Skand. Arch. Physiol., *72:* 73–77.

Initiation of Spike Potentials in Contact Chemosensory Hairs of Insects

III. D.C. STIMULATION AND GENERATOR POTENTIAL OF LABELLAR CHEMORECEPTOR OF CALLIPHORA[1]

HIROMICHI MORITA
Department of Biology, Faculty of Science, Kyushu University, Fukuoka, Japan

In the preceding studies of this series, the generation site of the impulses of the labellar chemosensory hair of *Lucilia* was localized at its proximal portion (Tateda and Morita, '59). From the study on the D.C. stimulation of the tarsal chemosensory hair of *Vanessa*, it was postulated that the D.C. current stimulated the chemosensory neuron at this generation site of the impulse (Morita and Takeda, '59). Now, it seems to be generally accepted that the local potential changes produced in sense organs by the various forms of stimuli initiate afferent impulses at the adjacent regions of nerve fibers (Granit's "generator potential," '47). Accordingly, from the above-mentioned results obtained in the insect chemoreceptors, it can be easily expected that the "generator potential" induced at the chemoreceptor surface gives rise to the impulses at the proximal portion of the chemosensory hair (cf. Dethier, '56). It is, however, none the less important to ascertain that it is really the fact.

In the present study, first of all, the effects of the D.C. current on the labellar chemosensory hairs of the fly were ascertained to be almost the same as mentioned above on the tarsal chemosensory hair of *Vanessa*. Next, the recording of the "generator potential" in the labellar hair was tried and resulted in good success. Further, it could be concluded from some experiments that we recorded the impulses which were "back-fired" along the distal process of the chemosensory neuron toward the hair tip.

MATERIALS AND METHODS

Most of the results in this study were obtained from *Calliphora vomitoria*, while only a few results were taken from *Lucilia caeser*. In this paper, therefore, without any special notice of the material, the experiments will be meant to be performed on the former. The proboscis of the fly was isolated and a platinum wire was inserted into it from the cut end. The tip of a chemosensory hair on this labellum was brought into contact with a test solution in a capillary into which another platinum wire was dipped. Then the potential difference of several hundreds milli-volts was detected between the two platinum electrodes. Accordingly, a potential source was additionally inserted for balancing this potential difference, though the circuit for D.C. stimulation was almost the same as that in the previous study (Morita and Takeda, '59; fig. 1). Designation of polarity of all the potentials to be added is the same as in the previous paper; i.e., the potential which makes the current flow from the outside to the inside of the chemosensory hair is described as *plus*, *anodal* or *anelectrotonus*, and the reversed polarity as *minus*, *cathodal* or *catelectrotonus;* only for the calibration, the pulse which makes the recording part more positive is designated as *plus*. The concentrations of the component solutes are indicated as final molar concentrations, in the same way as in the

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previous paper: A solution expressed as M/2 sucrose + M/4 NaCl, for example, contains ½ mole sucrose and ¼ mole NaCl per liter solution.

Impulses and slow potential changes can be recorded from the side wall of the chemosensory hair as follows: A glass-capillary c_1, one end of which is tapered to about 30 μ in diameter, is filled with an M/8 NaCl solution. This solution is connected through a platinum wire to the grid of the head-stage of a D.C. amplifier. Another capillary c_2 is drawn out at one end to the same diameter as that of a microelectrode used for impaling cells. Under a binocular microscope, with the aid of micromanipulators, a chemosensory hair is touched and supported on one side with the capillary c_1; at the very same level of the hair, the other capillary c_2 is pierced through the hair from another side. Under this preparation, the spontaneous impulses of chemosensory cells or the deflection of the beam due to the calibration pulse are observed. In this state, a test solution from the third capillary is applied to the tip of the hair. Then, impulses of characteristic patterns can be recorded with various kinds of solutions.

The grid current of the head-stage of the amplifier was in the range of 10^{-12} amp. The Braun tube used had a pair of deflection plates, the time axis of which had some angle to a horizontal axis. These sloped base-lines of repeated sweeps were photographed into lines spaced horizontally on a film running with an appropriate speed. For study of slow potential changes, the output-stage of the D.C. amplifier was comprised of an electronic switch; one of the divided beams served as the control base-line, and the other deflected according to the potential changes in chemosensory neurons.

RESULTS

D.C. stimulation from the hair tip[2]

Tips of a number of chemosensory hairs located on different labella were brought into contact with test solutions under various strengths of electrotonus which were applied between the two recording electrodes through a grid leak of 50 megohms, and impulses were recorded. One of the results is presented in figure 1. Because of the initial disturbance of the base-line, each histogram in this figure was obtained from the record for ¼ second immediately after the initial ¼ second during application of an M/8 NaCl solution under respective strength of electrotonus. Only one group of small spikes (1st group) were recorded under electrotonus below –0.05 v. Spikes belonging to a second group were additionally observed under electrotonus above –0.025 v, and, moreover, a third group of large spikes were added under electrotonus above +0.025 v. From this figure, it can be seen that the spike increases in number and decreases in height (except for 3rd group) with the increase in the anodal polarization. The number and height of spike (in mean value) are represented by figure 2 as functions of electrotonus. The groups of spikes other than the first are omitted as for the spike height, because they are in some cases too small in number to calculate mean values. The spike height of the

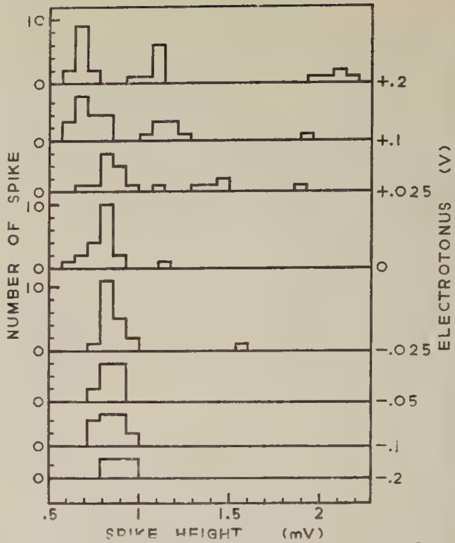

Fig. 1 Distributions of the spike height under various strengths of electrotonus which were applied to the hair tip through M/8 NaCl. The strength of electrotonus is indicated to the right of the base-line of each histogram. 23°C.

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[2] Important paper dealing with D.C. stimulation of labellar chemosensory hairs of the blowfly (Wolbarsht, '58) could be available after the completion of this work. The main results described in this paper well coincide with those reported under the present topic.

irst group is shown to be related linearly o the strength of electrotonus, being decreased with increasing anodal polariza-ion. On the other hand, the number of pikes of all the groups is indicated clearly o increase with an anodal electrotonus. The distribution of closed circles which epresent the number of spikes of the first group may be regarded as fluctuation around the line which is exponential under the catelectrotonus and horizontal under the anelectrotonus. The third group of spikes in figure 1 does not show any tendency as mentioned above. This group of spikes may not be considered to appear as a result of superposition of the two spikes belonging respectively to the first and the second groups. It is because, under the electrotonus of +0.2 v, the spike height in the first and the second groups is approximately 0.7 and 1.1 mv respectively, while the height in the third group is about 2.1 mv; namely, the height of the third group of spikes is too high, even if it is assumed that the peak of a spike of the first group was superimposed just on that of a spike of the second. The third group of spikes might be initiated in the neuron specialized to mechanoreceptor.

In a few of the experiments, a train of spikes of reversed polarity (i.e., the hair tip representing an increase in negativity with reference to its base) was initiated by catelectrotonus stronger than 0.2 v. As will be discussed later, this type of spikes is not considered to be conducted to the nervous center, even though they might occur elsewhere in the chemosensory neuron.

The records showing the same character as above are presented in figure 3. Electrical pulses of various *stimulus voltage* of approximately 0.15 seconds were applied while the hair was kept in contact with solutions, the bridge circuit being used as shown elsewhere (Morita and Takeda, '59, fig. 1a): just before the end of the electrical stimulation, the calibration pulse of +1 mv was added. In order to balance the difference between electrode potentials, a *balancing voltage* was produced before the test solution was administrated. The test solutions used were M/8 NaCl in record A, and M/4 sucrose + M/4 NaCl in records B and C. The *balancing voltage* in A, B and C were +0.5, +0.4 and +0.5 v, respectively. The upper and the lower records of each pair were obtained respectively when the *stimulus voltage* was −0.1 and +0.1 v. The train of spike potentials of a single type was recorded upon applying M/8 NaCl (record A), while the trains of two types of spikes were obtained in the case of M/4 sucrose + M/4 NaCl (records B and C). The histogram analysis as before indicated clearly that there appeared one group of spikes (about 1.5 mv) on the application of M/8 NaCl and two groups of spikes (about 1.5 and 0.8 mv, respectively) on the application of M/4 sucrose + M/4 NaCl. Therefore, it can be concluded that the train of small spikes (about 0.8 mv) occurred responding to sucrose, and also that the train of spikes in record A, as well as that of large spikes in records B and C, (both about 1.5 mv) represent the response to NaCl. It is dif-

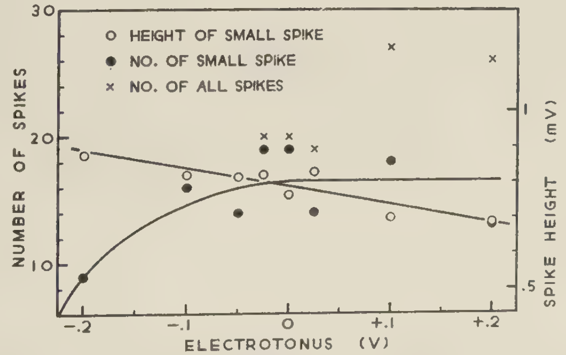

Fig. 2 Occurrence frequency and height of spike against the strength of electrotonus under which the spikes were obtained. Plotted from the result of figure 1.

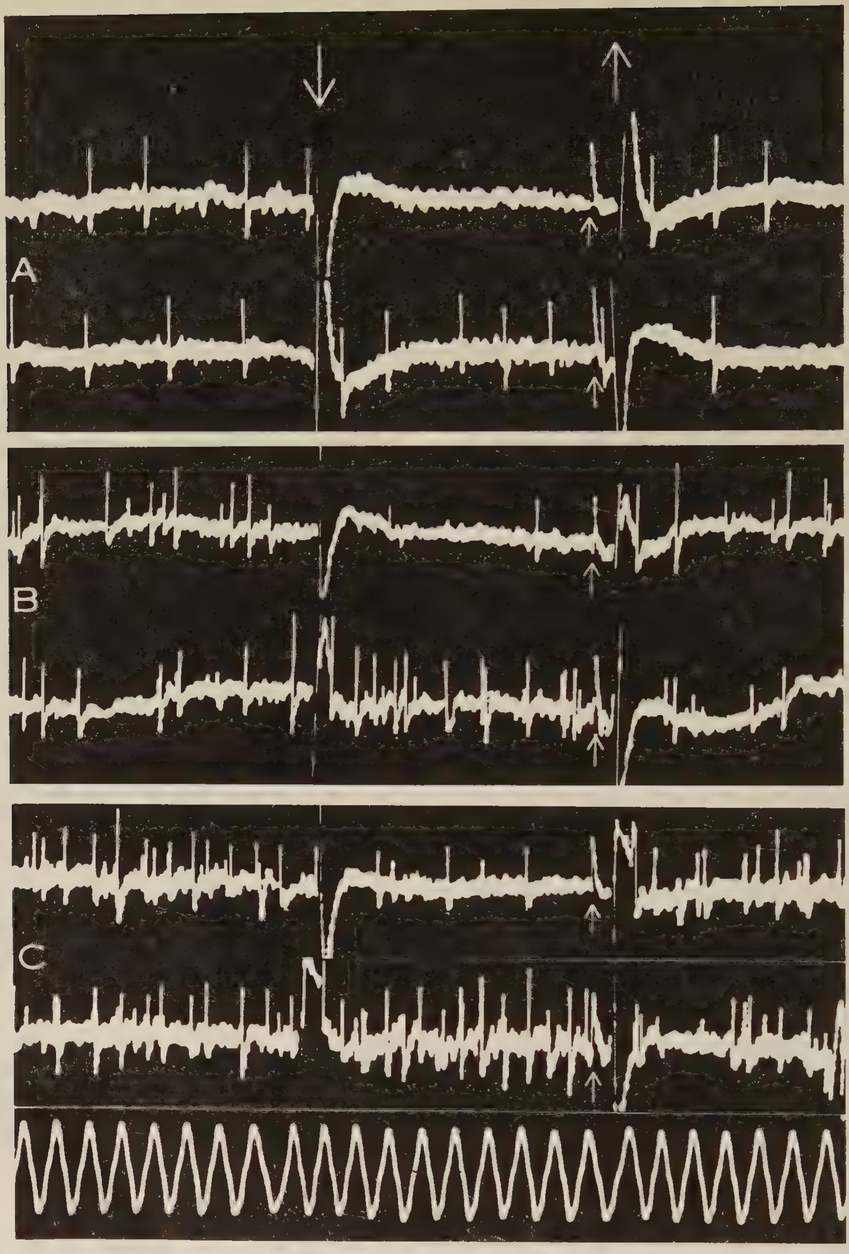

Fig. 3 Effect of electrotonus on the spikes responding to NaCl and sucrose. Arrows at the top, ↓ and ↑, indicate the start and the end of electrotonus, respectively. Small arrow in each record indicates the calibration pulse of + 1 mv. In each coupled records, cathodal and anodal pulses of 100 mv were added in upper and lower records, respectively. Test solutions and *balancing voltage* (see text): A, M/8 NaCl and + 0.5 v. B, M/4 NaCl + M/4 sucrose and + 0.4 v. C, M/4 NaCl + M/4 sucrose and + 0.5 v. Reconstructed from the same type of continuous record as figures 4, 5 and 6. Time, 1/60 second. 24°C.

ficult to decide whether each spike represents response to sucrose or NaCl; but it is seen clearly in this figure that the catelectrotonic or the anelectrotonic potential, respectively, inhibits or facilitates the occurrence of spike potentials of both types.

As indicated above, the results of D.C. stimulation of the labellar chemosensory hair of the fly are similar to those obtained in *Vanessa* preparation except in one point. That is, a few labellar hairs of the fly responded to cathodal polarization with spikes of polarity reversed to the ordinary chemosensory impulses. The electrical current flowing through the inside of the chemosensory neuron may flow out of this neuron somewhere between the tip and the base of the hair. This is possible in the case of labellar hair, for it is far longer than the tarsal chemosensory hair.

D.C. stimulation from the side wall of the hair

As described in the methods section, the electrical activity of the chemosensory neuron can be recorded from the side wall of the hair. Here also, the impulse represents an increase in positivity at the recording point with reference to the base of the hair. The records obtained thus are considered to represent the normal activity, because the reproducible and specific pattern of impulses are observed according to chemical stimulations for a long period as in the case of an intact hair. However, it seems that there are some precautions to take in application of this method. First, there are chances to injure mechanically the chemosensory neurons. This is easily detected by observing a burst of impulses during the operation of piercing the hair, and this occurs most frequently in the case of an inadequate size of the operating microcapillary. Secondly, the capillary electrode used for recording from the hole of the hair wall seems to be necessary to contain the salt solution of appropriate concentration. The concentration of M/8 of NaCl was determined empirically to be satisfactory. If a NaCl solution of high concentration, such as 1 M, is made to contact the hole, the impulse discharge begins a few seconds after the contact; the size of impulse is reduced, and, after several repetitions of contact, the chemosensory hair loses, at last, such a sensitivity. The mode of impulse discharge is shown in figure 4, where the record continues from A to D. One M NaCl was brought into contact with the hole at the proximal level of the hair and the impulses were recorded from the distal hole through an M/8 NaCl solution. After a fairly long period from the moment of the contact of 1 M NaCl (indicated by the transient upward deflection at the top in A), the impulse begins to occur in a very low frequency, but the frequency thereafter increases more and more. Really, it seems as though we see the diffusion process before our eyes. The heights of spikes are smaller than the ordinary chemosensory spikes, and it should not be assumed that the part of the neuron on the half way of the hair has the same chemoreceptive properties as at the tip. In fact, when a M/4 sucrose solution was brought into contact with the proximal hole, no train of impulses resulting from this contact was recorded from the distal hole. In one of the hairs, the recorded spontaneous spikes increased in amplitude two to three times as large as those obtained before the application of sucrose from the side hole. This may be attributed to an insulating effect of the sucrose solution.

It was generally observed, that a stimulation with an anodal current was possible even when the current was applied to a hole located on the side wall of the hair, and impulses were recorded from the same hole. Such impulses were, in some cases, blocked by $CaCl_2$ and quinine hydrochloride applied to the hair tip. On the other hand, at times, a brief burst of impulses could be recorded during the initial period of cathodal pulse. In this case, the polarity of the impulse was the same as that of the ordinary chemosensory impulse, and the change in shape also was hardly detectable. Even when the cathodal stimulation was thus possible, the anodal current, after some delay, increased impulses more than the cathodal one; i.e., impulses were increased in number more and more after the anodal stimulation started. This reminds us of the case of the application of 1 M NaCl to the

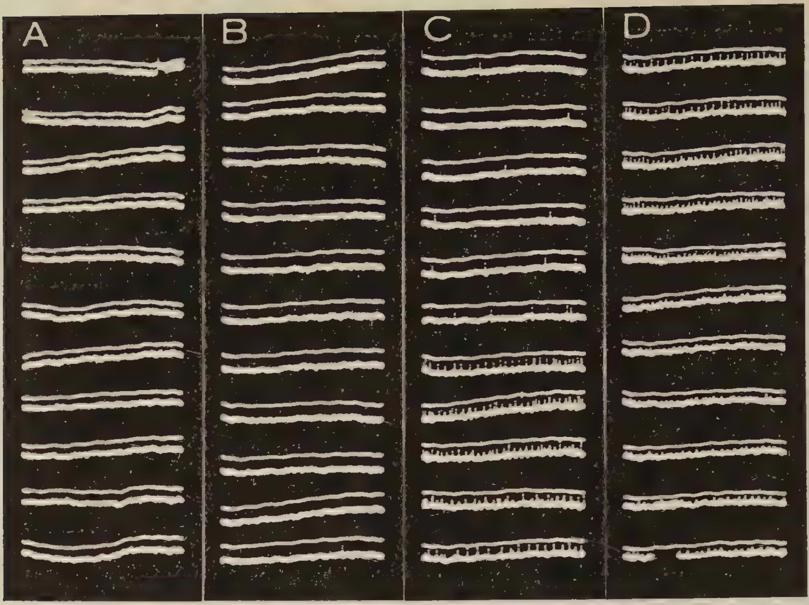

Fig. 4 Spikes initiated when 1 M NaCl was applied to the hole pierced through the hair wall. Record continues from A to D, with the control base-line on the upper side. The deflected beam due to + 1 mv of calibration is seen to be superimposed on the second sweep from the bottom of column D. One sweep, about 1/6 second. 28.6°C.

hole of the hair wall. Consequently, it may be assumed, at least in some cases, that the cathodal current from the hole in the wall stimulated the chemosensory neuron electrically, whereas, the anodal current stimulated it ionophoretically. The results of the electrical stimulation from the hair tip, which are somewhat different in this case, can be understood, because most parts of the current applied to the tip were expected to flow within the chemosensory neuron.

Generator potential

In the previous paper (Morita and Takeda, '59), it was shown that the slow potential change resulted from bending the hair, and that this was related to the initiation of impulses. From the beginning of our study of chemosensory hairs of insects, we tried to record the generator potentials of chemosensory neurons from the hair tip. First, the trial was undertaken by adjusting the potential of the stimulus solution, and the distortion of the base-line during an initial period of contact was depressed as small as possible. This method made several repetitions of contact of the solution with the hair tip necessary in order to determine the balancing voltage. Therefore, the hair was adapted to the solution when the precise adjustment was attained, and although the slow potential changes could be obtained in this way, they did not show any regular correlation to the sensory impulses. The second method was as follows: The hair tip was connected steadily to the electrode through an M/8 NaCl solution, and the stimulus solution contained in another capillary was brought into contact with the M/8 NaCl solution, with the expectation that the stimulus substance would diffuse to the hair tip. Then, the potential of the solution in the capillary electrode was increased in positivity when a sucrose solution was used as the stimulus. When the NaCl solution in the capillary electrode had not been kept in contact with the hair tip, no potential change was induced by bringing the stimulus solution into contact with the electrode solution. Therefore, such a potential change as recorded above can not be considered to be an artifact due to a leaky current, but it should be assumed to be a result of an interface phenomenon occurring between the stimulus solution and the medium in the hair tip. However, the magnitude of this potential change was too large in comparison with chemosensory impulses to be related to the initiation of the chemosensory impulses. Thus, the generator potential could be recorded only from the hole in the side wall of the hair.

The records from the side wall of one chemosensory hair by means of the direct amplifier are shown in figures 5 and 6. The records were obtained in the order from A in figure 5 to D in figure 6. In record 5A, the instant of the contact of the M/4 sucrose with the hair tip can be recognized on the way of the third sweep by a brief upward (positive) deflection of the beam, which should be considered as a contact artifact. Following this, the sustained downward (negative) deflection is seen, being accompanied by trains of impulses. The time course of this negativity is not accurately represented by this record because of the somewhat inconstant speed of the film and because the sweep was not exactly linear. If necessary, however, the time course can be estimated correctly from the departure of the beam from the control base-line. Immediately after application of M/4 sucrose, impulses are seen to be discharged most intensely and the slow deflection attains a maximum. Thereafter, the impulse is decreased in the occurrence frequency and also, though to a much less extent, the sustained negativity is decreased in amplitude. Contrary to the initial contact of the test solution, a drastic artifact is not seen on withdrawal of the test solution. In this experiment, the square pulse of + 1 mv for calibration was added after the withdrawal of stimulant, and the deflection of this pulse is seen on the last sweep in this record. The instant of the withdrawal should be identified on some sweep other than the last one. Thus, it is safe to assume that the point on the 8th sweep where the base-line turns upwards corresponds to the instant of the

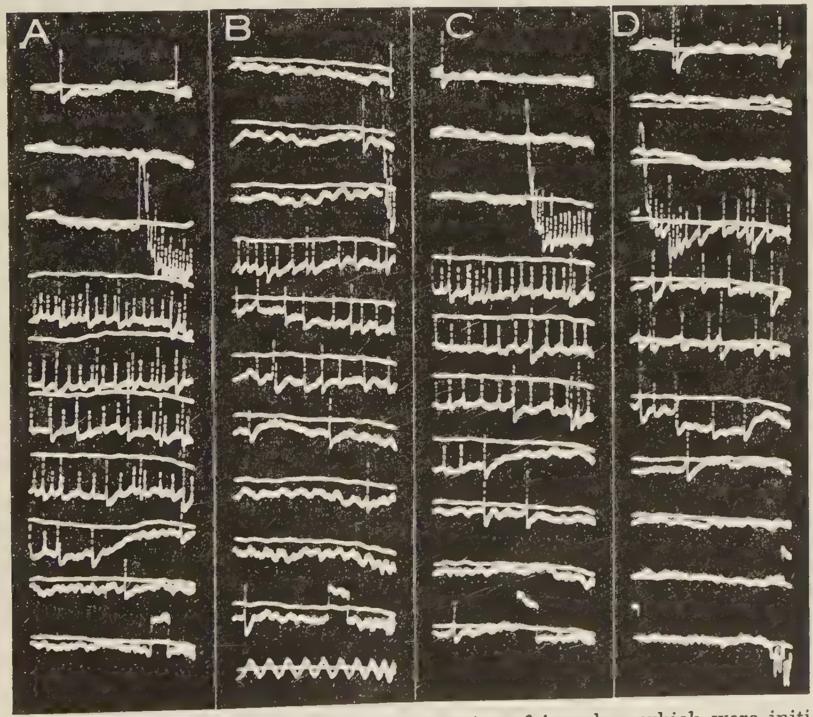

Fig. 5 Sustained negativity accompanied by trains of impulses which were initiated by chemical stimuli. A, M/4 sucrose. B, 1 M NaCl. C, M/16 sucrose. D, M/4 NaCl. Time, 1/60 second. Calibration pulse, + 1 mv. 19°C.

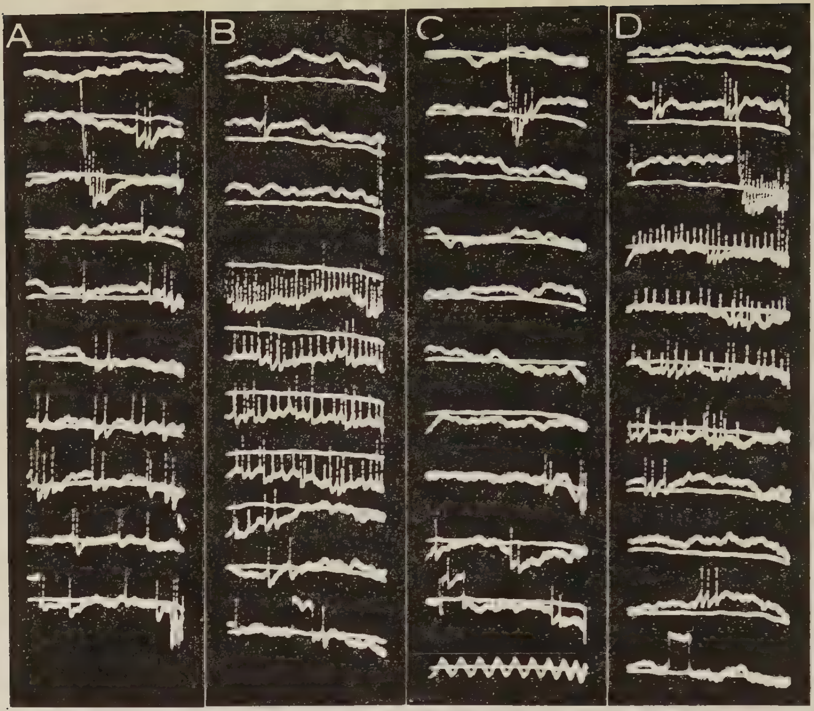

Fig. 6 Continued from figure 5. A, M/4 sucrose + M/640 quinine hydrochloride. B, M/4 sucrose. C, M/4 sucrose + M/160 quinine hydrochloride. D, M/4 sucrose. All other indications are the same as in figure 5.

withdrawal. After this point, only two impulses are seen before the calibration pulse; such a rate of impulse discharge is seen also on the first sweep before the application of M/4 sucrose. In record 5B, the onset of the application of 1 M NaCl is indicated by the contact artifact immediately before the end of the third sweep, and is followed by the slow downward deflection of base-line and the discharge of impulses. Compared with the case of M/4 sucrose, the depth of the sustained deflection is shallower and the occurrence frequency of impulse is lower. Accordingly, the return to the resting level of the base-line on withdrawing the 1 M NaCl solution is not as clearly seen as in the case of M/4 sucrose; but the point corresponding to the withdrawal can be localized at the point immediately after the start of the 7th sweep. After this point, the base-line maintains its position on the same level as that before the stimulation. Record 5C was obtained on stimulating the hair by M/16 sucrose. Its general feature is completely the same as that of record 5A, except for the decreases in the frequency of impulse and in the depth of the sustained downward deflection. This is quite reasonable because the stimulus strength was weak in this case. Record 5D resulted from the stimulation of the hair by M/4 NaCl. The effect of this stimulation is much less than in the above-mentioned cases, but the general feature can be still detected. When the hair was stimulated by M/4 sucrose + M/640 quinine hydrochloride, a quite different feature of record was obtained (record 6A). As before, the initial contact artifact is followed by the burst of impulses and the slow downward deflection, but, in this case, both of them disappear in a short period. As mentioned above, the test solution was certainly withdrawn before the calibration pulse (this is seen to be separated at the end of the 9th and the beginning of the 10th sweep); nevertheless, impulses are suddenly increased in the rate of discharge on the 7th sweep and this rate of discharge seems to be decreased gradually but is maintained without any sudden drop even after the calibration pulse. The instant of the withdrawal of the test solution may be localized elsewhere on the 6th or 7th sweeps; because it can be detected somehow that the level of the base-line is lowered, and the after-discharge of impulses is frequently observed in the case of stimulation by inhibitors. After it was ascertained that the hair responded normally to M/4 sucrose (record 6B), the higher concentration of quinine in the sucrose solution (M/4 sucrose + M/160 quinine hydrochloride) was administrated to the hair (record 6C). In this case, the beam rises from the peak of negativity more steeply than in record 6A, and arrives at the level which is more positive than before the stimulation. The maintenance of this positivity during the stimulation seems to be masked by irregular fluctuations of the base-line, but the several records obtained from other preparations indicated this maintenance. In parallel with this maintenance of positivity, no impulse is seen before the 8th sweep, on the way of which the test solution is considered to be withdrawn. After this test, the hair was stimulated again by M/4 sucrose to check the normality in response (record 6D). Thus, the beam is deflected downwards after the application of M/4 sucrose and sustained to the point before the end of the 7th sweep.

Recorded from the more distal part of the hair, the sustained negativity seems to be the larger in amplitude. When the record was obtained at the level of approximately a third of the hair length from the tip, the persistent parallelism between the sustained negativity and the train of impulses such as shown in figures 5 and 6 could be seen in all the hairs tested. This is, first of all, the reason why the sustained negativity is regarded as the generator potential of the chemoreceptor. It might be wondered why the "generator potential" was sometimes recorded to be larger than the initiated spike potentials; for the "generator potential" is at most the depolarization of the membrane potential, while the spikes generally overshoot it. This question can be resolved as follows: (1) Many "generator potentials" of other receptors which have been recorded occur on the same side as spikes with reference to recording electrodes. In the present case, however, as will be discussed later, the one of the recording electrodes was imposed between the loci of the generator and the spike potentials. (2) Even the stimulation by a plain sucrose solution initiated two trains of impulses (fig. 5A and C). Therefore, the generator potentials of two chemosensory neurons must be recorded simultaneously, and the generator potential of the single neuron may have been smaller than seen in the records. It might also be wondered why some impulse trains were recorded when sustained negativities were hardly detected (fig. 5D and 6A). Unfortunately, the records were accompanied by the fairly large fluctuations which seemed to be due to the change in contact of the recording electrode with the medium within the hair. Accordingly, the fluctuations may have masked the sustained negativity just below the resting level. Judged from the magnitude in negativity as seen in figure 5B where the hair was stimulated by 1 M NaCl, it is rather reasonable that the negativity was as small in amplitude as it was hardly detected in figure 5D where the hair was stimulated by M/4 NaCl. It may be assumed that the magnitude of the generator potential and the frequency of impulse on sweeps after 7th in figure 6A were in the same orders as on 5th and 6th sweeps in figure 5D.

The initial positive surge at the onset of the stimulation was considered before as an artifact without any explanation. This is represented by the results shown in figure 7. Record a in this figure A was obtained as before by stimulating a chemosensory hair by M/16 sucrose. The beam representing the activities of the chemosensory neurons has its position fairly above the control base-line before the stimulation. After the contact of the test solution, the beam is seen to drop to nearly the same level as the control base-line. After record a was obtained this hair was mechanically injured at the recording point, and then stimulated by M/4 sucrose (record b). In this record,

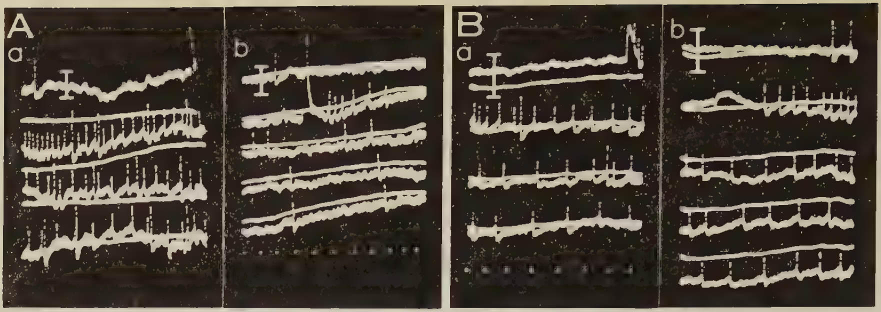

Fig. 7 A, the simultaneous diminution of chemosensory impulses and the sustained negativity after the neuron was injured mechanically at the recording part of the chemosensory hair. a, before the injury; stimulated by M/16 sucrose. b, after the injury; stimulated by M/4 sucrose. 16°C. B, Diminution of the initial artifact when the same chemical stimulation (M/4 sucrose) was applied successively. Record b was obtained immediately after record a. 23°C. Calibration, 1 mv and 1/60 second.

the sustained negativity is negligibly small in amplitude and the impulse is greatly reduced in number, while the initial surge becomes rather large. Therefore, it is concluded that the initial positive surge is some artifact due to a physical phenomenon evoked at the surface of the hair tip; on the other hand, the sustained negativity, as well as the train of impulses, is postulated to be a physiological event. Another evidence is the result presented in figure 7B. Immediately after record a was obtained by stimulating the hair with M/4 sucrose, the hair was again stimulated in completely the same way (record b). The initial surge is clearly shown in the first record, but it is not in the second one. Accordingly, this initial artifact is assumed to be due to something such as a potential difference which may have existed, before the contact, between the surfaces of the hair tip and the test solution. If the records such as figures 5 and 6 were obtained without the initial artifact, it would be indicated, as is the case in record b of figure 7B, that the discharge of impulses started at the potential just below the resting level.

Transmission of the generator potential

Since the main purpose of this paper is to explain how the impulse is initiated in the chemosensory hair, it is sufficient here to note that the generator potential arises in the chemoreceptor and its polarity is negative at the recording point with reference to the hair base. The various features of the generator potential itself will be reported in another paper.

In the preceding paper, it was shown that impulses originated at the basal region of the chemosensory hair (Tateda and Morita, '59), and, now, the generator potential could be recorded. It is concluded from its polarity that the generator potential is the depolarization of the chemoreceptive surface membrane which is located at the hair tip. This depolarization may spread electrotonically to the proximal part of the chemosensory neuron and initiate impulses there (fig. 11). However, it might be assumed alternatively that the generator potential initiates, near the hair tip, the impulse which is too weak to be recorded; this impulse might be conducted to the basal region and grow up to the impulse strong enough to be recorded.

Of the two alternatives, the former assumption may be acceptable, because the electrotonic current which is recorded to be as strong as chemosensory impulses from some levels of the hair may be sufficient in strength also for initiating impulses at the site near the hair base. Concerning this problem, the results represented by figures 8 and 9 may be decisive. A labellar chemosensory hair of *Lucilia caeser* was cooled partially with the sharpened tip of the copper wire which was in turn cooled with dry ice on the other side, and impulses were recorded from the hair tip. First, the impulse was

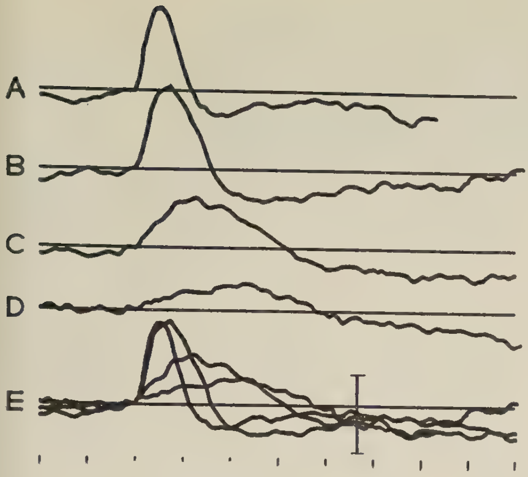

Fig. 8 Cooling effect on the shape of spike. A, before cooling. B, effect of cooling at a part departed from the hair base. C, initial period of cooling at the hair base. D, later period of the same as in C. E, all the above 4 are superimposed. Labellar chemosensory hair of *Lucilia*. Calibration, 1 mv and one millisecond. 20.5°C.

recorded without cooling (trace A in fig. 8). Secondly, the tip of the cooling wire was touched at the middle part of the hair. Thus, record B in this figure was obtained. Thirdly, the base of the hair was touched with cooling wire tip at the surface of the labellum. Thus, record C and, thereafter, record D was obtained. In E, all the 4 traces are superimposed, being based on the time axis of the oscilloscope and the rising feet of the spikes. The effect of cooling the hair at its middle part is seen on the falling phase of the impulse, but little or none on the rising rate and the height of spike. The lowering of the spike height and the rising rate is seen clearly only when the hair was cooled at its base. The same kind of result from another preparation is shown in figure 9: The record of trace A was obtained without cooling, and the record B by cooling the hair at the part some distance away from the hair base; the record C was obtained during the initial period of cooling the hair at its base and the record D during the later period; in E, all the 4 traces are superimposed. In this case, the height and the rising rate of the spike was lowered a little, but the falling rate much more, by cooling the hair at the middle portion (trace B); on the other hand, the effect on the falling phase was much more drastic than on the rising phase of the spike upon cooling the hair at the base (trace C). The temperature at which the hairs were cooled was below 0°C, the crystals of ice being formed there; the effect of the cooling was completely reversible and reproducible. From this result, it should be concluded that the chemosensory impulse which is recorded more or less diphasically is composed of the two components, the positive and the negative spikes; the negative spike represents the activity of the distal part of the chemosensory neuron which is more affected by the cooling of the hair, while the positive spike represents the proximal activity which is less affected by the cooling. This must be a result of the back firing from the impulse-initiation site which has been localized somewhere near the hair base. If the impulses were initiated at a region adjacent to the chemoreceptive surface and conducted proximally, the neuron would discharge impulses of different strengths at the same point, the orthodromic being invisibly weak and the antidromic being strong. This is quite inconceivable from the all-or-none principle of the conducted impulse.

Figure 10 shows the various forms of the chemosensory impulses which were obtained frequently in the summer season from deteriorated chemoreceptors. The small impulses shown before in figure 5A

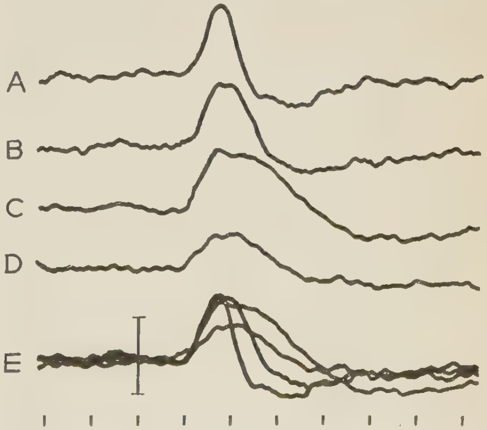

Fig. 9 All the same as in figure 8, but the slowing down of falling relative to that of rising rate is much more drastic on cooling the hair base (C). 21°C.

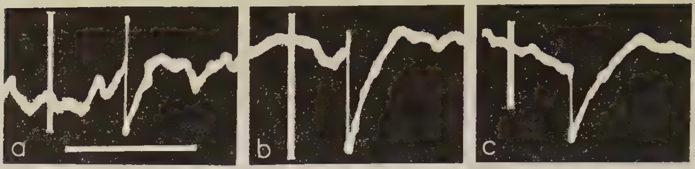

Fig. 10 Various shapes of spikes which were recorded from somewhat deteriorated materials. Calibration of amplitude, 0.5 mv in a; 1 mv in b and c. Time, for all records, 1/100 second. 28°C.

represent one extremity of the positive monophasic spike, and record c in figure 10 represents the other extremity of the negative; other records are the intermediate forms. Those forms of impulses may have resulted from the relative amplitudes of the above-mentioned two components. Among them, record c is specially interesting. The fact of the back firing from the initiation site of impulse means that the chemosensory neuron is able to discharge impulses at a part distal to the initiation site, though the threshold there is much higher than at the latter. This implies that, under certain circumstances, impulses can be initiated at the distal part without the back firing. Record c is considered to be this very case: Due to deterioration, the neuron at the part where impulses were initiated normally may be depolarized and not initiate impulses any more. On the other hand, the neuron at the distal part may be under the influence of the strong cathodal current from the regions of this depolarized "initiation site" and of the hair tip, and initiate impulses by itself. This may have resulted in the negative monophasic spike as record c. The impulses of reversed polarity as described before, when the strong cathodal current was applied to the hair tip, could be explained from essentially the same principle. In this case, the "initiation site of impulse" may be under the condition of the anodal block, and the strong cathodal current may initiate impulses at the region distal to the "initiation site." Furthermore, the deformation of impulse induced by bending the hair (Morita and Takeda, '59) can be also considered as a result of the lowering and slowing down of the distally conducted spike. With respect to this point, the following observation would be suggestive: Impulses were recorded simultaneously from the tip and the hole in the side wall of a labellar hair. The hair was bent, with the electrode on the tip, against the electrode on the hole. Then, spikes recorded from the tip were distinctly prolonged in their falling phases, compared with those recorded from the side.

DISCUSSION

General feature of the chemoreception

From the same reasons as given in the preceding paper of this series (Morita and Takeda, '59), the following can be concluded: Impulses are initiated by the cathodal D.C. current at the "initiation site" of chemosensory impulses; the site is localized somewhere near the hair base (Tateda and Morita, '59). Upon the ordinary chemical stimulation, the generator potential at the chemoreceptor surface may play the role of the source of this cathodal current. Thus, the general description of the contact chemoreception of insects is possible as illustrated schematically in figure 11. For brevity, only one cavity as well as only one neuron are drawn in the hair. When chemical stimulants are brought into contact with the tip of the chemosensory hair, the membrane of the chemoreceptive surface (the shaded area a of figure 11A) is depolarized. Then, current flows along the course indicated by arrows in the space outside the neuron. The potential drop due to this current is recorded as the sustained negativity with the electrode kept in contact with the space outside the neuron. On the other hand, this current initiates impulses in the neuron at the site near the hair base (area b). The additional currents due to these impulses flow along the thin arrows in figure 11B; the potential drops due to these currents are recorded, with the same electrode, as the increases in positivity superimposed upon the sustained negativity.

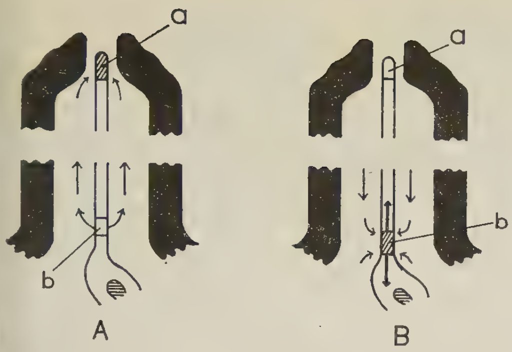

Fig. 11 Scheme of chemosensory hair to illustrate the chemoreception. Shaded areas represent the active regions of the chemosensory neuron. A, the generator potential. B, chemosensory impulses. a, the chemoreceptor region. b, the initiation site of impulses. Thick arrows represent the direction along which impulses are conducted; thin arrows, current-flow.

The impulses are, of course, conducted proximally to the nervous center, and also distally towards the hair tip (indicated by the thick arrows). Thus, the distally conducted impulse makes the recorded impulse form briefer than the original one of the initiated impulse, and this generally results in more or less the diphasic impulse.

As to the polarity of the impulses recorded from the chemosensory hair, the quite contrary results were reported by Hodgson and Roeder ('56) and by Morita et al. ('57). Namely, the former described the impulses representing an increase in negativity at the tip with reference to the base of the chemosensory hair, while the latter recorded those of the reversed polarity. Hodgson ('58) has recently been able to trace the contrary reported before to an error in instrumentation; he observed the impulses recorded as positive spikes at the tip of the chemosensory hairs of the various species of arthropods. Thus, it might be possible that the feature of chemoreception presented here holds generally for the chemosensory hairs of arthropods.

Generator potential

As Granit described in his monograph ('55), the mechanism of initiating impulses at nerve endings seems to be run on similar principles throughout all kinds of receptors. That is, an adequate stimulus induces the local potential change (the generator potential) in a receptor, and the electrotonic spread of this potential change sets up impulses in the neuron at a region adjacent to the receptor. The generator potential, in fact, has been recorded from the Pacini body (Alvarenz-Buylla and Ramirez de Arellano, '53; Gray and Sato, '53), and from the muscle spindle (Katz, '50). The cochlear microphonics are now held to be the generator potentials of the mechanoreceptors in the ear (Davis, Tasaki and Goldstein, '52); the electroretinograms, at least some components of them, are considered to be the generator potentials of the photoreceptors of the eye. Above all, the slowly adapting lobster stretch receptor shows the similarity to the insect chemoreceptor presented here to the full extent: Eyzaguirre and Kuffler ('55a, b) have recorded the generator potential of this stretch receptor; more recently, Edwards and Ottoson ('58) studied on the site of impulse initiation in the nerve cell of this receptor, and could localize it at a part which was as far as 500 μ proximal to the cell body. Furthermore, it has been clearly shown that the initiated impulses are conducted backwards to the cell body. Thus, the back-firing of impulses concluded in the present paper is not so peculiar.

Record of conducted impulses

In this paper, it was assumed that records were obtained with the electrode which was, at a certain level of the labellar hair, kept in contact with the medium suspending the chemosensory neurons. However, as Grabowski and Dethier ('54) have shown in the chemosensory hairs of *Phormia*, the labellar chemosensory hair of *Calliphora* also has two cavities, only to one of which the processes of the neurons are supplied. The other cavity is larger and enclosed by a thinner wall. Therefore, the chance to be brought into contact with this large cavity only is expected to occur fairly frequently. Whether or not the activities of the chemosensory neurons can be recorded in this state is not decided from the present experiments; it is because the hair was discarded when any activity was not recorded, though the conductivity was established between the inside of the hair and the electrode contact with the side wall of the hair. If it is assumed that the activity of the neuron could be recorded from the outer cavity including no neuron, it must be assumed that the records represented the potential changes at the region in the labellum, where the outer cavity is shown to open. This is considered from the fact that the outer cavity is insulated from the small cavity containing neurons by the thick wall within the hair. However, it may be sure that the impulse is conducted distally within the hair, judged from the instant effect of cooling at the part departed from the hair base. Hoyle ('55) succeeded in recording the impulse of the fiber of 8 μ in diameter within the *Locusta* ocellar nerve of the diameter of 65 μ, though the drying up of the nerve was necessary. The ratio of the neuron diameter to that of the cavity enclosing the neuron is in a range 1/5 to 1/3 (unpublished data of the electronmicroscopical study of Tominaga), and, therefore, it is not surprising that one can record impulses of the neurons under such circumstances.

Mechanism of the peripheral inhibition

Tateda ('59) reported the peripheral inhibition by quinine and acetic acid in the chemosensory hair of *Lucilia* labellum. Hodgson ('57) described the same sort of inhibition by heavy metals in the labellar chemosensory hair of the blow-fly. Morita and Takeda ('59) indicated also the direct inhibition by calcium in the tarsal chemosensory hair of *Vanessa*. It was clearly shown in this study that the inhibition by quinine resulted from the hyperpolarization which antagonized the generator potential. In this case, this hyperpolarizing effect was seen to be preceded by the brief initial depolarization due to sucrose. On the other hand, the inhibiton by acetic acid was observed to reduce the magnitude of the depolarization due to sucrose as a whole. Thus, it may well be true that the effect of the direct inhibitor consists in antagonizing the depolarization of the generator potential.

The pattern of the impulse discharge evoked by applying M/4 sucrose + M/640 quinine (fig. 6A) seems to need some mentioning. The impulses discharged during the initial negativity are the same as those responding to sucrose, but the impulses occurring thereafter in low frequency are the same as those responding to NaCl or the spontaneous ones occurring before the stimulation, being judged from their strengths and forms. Quinine of higher concentration in the sucrose solution inhibits completely even these spontaneous impulses during its application (fig. 6C). Therefore, these impulses of low frequency observed in figure 6A might not be regarded as the specific response to quinine. As reported by Tateda ('59), quinine inhibits generally the initiation of both impulses responding to sucrose and NaCl. In some cases, however, though they might be abnormal, quinine initiated impulses by itself. Such an occurrence of impulses is quite irregular, so that the record could not be obtained on the succeeding application after the positive response to quinine was observed. The impulse initiation was reported on stimulating the tarsal chemosensory hair of *Vanessa* by quinine (Morita et al., '57), and the impulse pattern in that case was quite similar to that in figure 6A in the present paper. It is thus assumed that quinine initiates, under some circumstances, impulses in an insect chemoreceptor which is not specialized for detection of quinine.

SUMMARY

1. Labellar chemosensory hairs of the fly, *Calliphora vomitoria*, were stimulated by D.C. current through test solutions applied to the tip. The impulse of a chemosensory neuron is decreased in strength, being linearly related to the increase in the D.C. current anodal at the tip; while, under the same circumstances, its occurrence frequency could be assumed to be increased exponentially. In a few cases, the strong cathodal current initiated impulses of the reversed polarity.

2. The hairs were stimulated in the same way as above, but at the hole pierced through the wall of the hair. Generally, the anodal current increased the occurrence frequency of impulse, while the cathodal one decreased it. Calcium and quinine applied to the hair tip blocked the impulses induced by this stimulation. In some cases, the cathodal stimulation induced a brief burst of impulses. Even in

these cases, the frequency of impulses increased more and more after the anodal stimulation started.

3. The results mentioned above were explained by the same principle which was described in *Vanessa* (Morita and Takeda, '59). Namely, the electrical current is cathodal and effective at the initiation site of impulse, which was localized at the base of the hair (Tateda and Morita, '59).

4. The "generator potential" of the chemoreceptor in the hair was recorded. This potential represents an increase in negativity at the chemoreceptor region which is located at the tip of the hair, and is maintained during the chemical stimulation.

5. The effect of the cooling of the hair at the level departed from its base was much more drastic on the falling phase of the spike than on the rising phase. This indicates the existence of a distally conducted impulse from its initiation site. Any proximally conducted impulse within the hair was not suggested to exist.

6. In this way, the general feature of the primary events in the insect chemoreception was drawn as follows: The arrival of a chemical stimulant at the chemoreceptor surface of the neuron at the hair tip depolarizes the membrane there; this depolarization spreads to the basal region of the hair, and initiates impulses; the impulses are not only proximally conducted to the center of the nervous system, but also distally toward the hair tip. By this feature of chemoreception, many variations of impulse in shape could be explained.

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LITERATURE CITED

Alvarez-Buylla, R., and J. Ramirez de Arellano 1953 Local responses in Pacinian corpuscles. Am. J. Physiol., *172:* 237–244.

Davis, H., I. Tasaki and R. Goldstein 1952 The peripheral origin of activity, with reference to the ear. Cold Spr. Harb. Symp. Quant. Biol., *17:* 143–154.

Dethier, V. G. 1956 Chemoreceptor mechanism. Molecular structure and Functional activity of Nerve Cells. pp. 1–30. Amer. Inst. Biol. Sci. Publ. No. 1, Washington, D. C.

Dethier, V. G., and M. L. Wolbarsht 1956 The electron microscopy of chemosensory hairs. Experientia, *8:* 335–337.

Edwards, C., and D. Ottoson 1958 The site of impulse initiation in a nerve cell of a crustacean stretch receptor. J. Physiol., *143:* 138–148.

Eyzaguirre, C., and S. W. Kuffler 1955a Process of excitation in the dendrites and in the soma of single isolated sensory nerve cells of the lobster and crayfish. J. Gen. Physiol., *39:* 87–119.

——— 1955b Further study of soma, dendrite, and axon excitation in single neurons. Ibid., *39:* 121–153.

Grabowski, C. T., and V. G. Dethier 1954 The structure of the tarsal chemoreceptors of the blowfly, *Phormia regina* Meigen. J. Morph., *94:* 1–19.

Granit, R. 1947 Sensory Mechanisms of the Retina. Oxford Univ. Press, London.

——— 1955 Receptors and Sensory Perception. Yale Univ. Press, New Haven.

Gray, J. A. B., and M. Sato 1953 Properties of the receptor potential in Pacinian corpuscles. J. Physiol., *122:* 610–636.

Hodgson, E. S. 1957 Electrophysiological studies of arthropod chemoreception. II. Responses of labellar chemoreceptors of the blowfly to stimulation by carbohydrates. J. Insect Physiol., *1:* 240–247.

——— 1958 Electrophysiological studies of arthropod chemoreception. III. Chemoreceptors of terrestrial and fresh-water arthropods. Biol. Bull., *115:* 114–125.

Hodgson, E. S., and K. D. Roeder 1956 Electrophysiological studies of arthropod chemoreception. I. General properties of the labellar chemoreceptors of Diptera. J. Cell. and Comp. Physiol., *48:* 51–76.

Hoyle, G. 1955 Functioning of the insect ocellar nerve. J. Exp. Biol., *32:* 397–407.

Katz, B. 1950 Depoloralization of sensory terminals and the initiation of impulses in the muscle spindle. J. Physiol., *111:* 261–282.

Morita, H., S. Doira, K. Takeda and M. Kuwabara 1957 Electrical response of contact chemoreceptor on tarsus of the butterfly, *Vanessa indica.* Mem. Fac. Sci. Kyushu Univ. Ser. E. (Biol.), *2:* 119–139.

Morita, H. and K. Takeda 1959 Initiation of spike potentials in contact chemosensory hairs of insects. II. The effect of electric current on tarsal chemosensory hairs of *Vanessa*. J. Cell. and Comp. Physiol., *54:* 177.

Tateda, H. 1959 The electrical responses of the labellar chemosensory hairs of the fly to sucrose, sodium chloride, quinine hydrochloride, and acetic acid. (In preparation).

Tateda, H., and H. Morita 1959 Initiation of spike potentials in contact chemosensory hairs of insects. I. The generation site of the recorded spike potentials. J. Cell. and Comp. Physiol., *54:* 171.

Wolbarsht, M. L. 1958 Electrical activity in the chemoreceptors of the blowfly. II. Responses to electrical stimulation. J. Gen. Physiol., *42:* 413–428.

SUPPLEMENT

SOCIETY OF GENERAL PHYSIOLOGISTS

Abstracts of papers presented at the fourteenth annual Meeting,[1] University of Illinois, Urbana, Illinois, September 7, 8, and 9, 1959

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[1] Abstracts or summaries of programs of previous meetings will be found in Biol. Bull., 91: 236; 93: 222; 95: 281; 97: 267; 99: 308 (1946–50); Science, 114: 699 (1951) and 118: 768 (1953); J. Nat. Cancer Inst., 13: 1399 (1953); and J. Cell. and Comp. Physiol., 44: 327; 46: 353; 48: 329; 50: 349–356; 52: 189–193; (1954–1958).

ABSTRACTS

T. C. BARNES, Hahnemann Medical College, Philadelphia, Pa. *Phase-boundary potentials of phrenotropic substances in the oil-cell.*

Brodie (Fed. Proceed. 17: 1163, 1958) and Woolley (Fed. Proceed. 18: 461, 1959) have recently revived interest in oil-solubility of drugs affecting the nervous system. Adrenergic substances (influencing the ergotropic mechanism of Hess) establish phase-boundary potentials on triglyceride oils in contrast to agents like acetylcholine affecting the endophylactictrophotropic system (Barnes and Beautner, Fed. Proceed. 7: 204, 1948). Chlorpromazine, 0.05%, generates 50 mv. negative at a triacetin-saline interface and may be adrenolytic by competitive solubility inhibition. Ergotamine, a congener of LSD-25, also gives a potential on adrenergic oils. Serotonin, 0.05%, produces 10 mv. negative on triacetin-saline boundary and is probably not a parasympathetic transmitter. Reserpine, 0.002%, gives a potential of 15 mv. on an oleate membrane and is possibly active independently of catechol amine release. Meprobamate is unique in that it has no electrical action in the model. A possible mechanism of action is suggested by the following: KCL, 0.01%, generates 50 mv. negative on an oleate layer which is reduced to 25 mv. negative when 0.5% inert Meprobamate is suspended in the oil.

THOMAS A. BORGESE and JAMES W. GREEN, Rutgers University, New Brunswick, New Jersey. *The effect of rose bengal on glucose utilization in rabbit erythrocytes.*

Rabbit blood obtained by heart puncture was centrifuged, leucocytes were removed and the cells washed twice in 0.16 M NaCl buffered to pH 7.4 with 0.01 M phosphate. One percent suspensions of these cells in buffered isotonic NaCl were exposed to 2×10^{-6} M rose bengal and photosensitized by one minute exposure to light from a 15 W fluorescent bulb in a condenser-like irradiation chamber. The suspensions were subsequently concentrated to 10% cells and with the addition of glucose incubated at 37°C for periods up to 6 hours. The disappearance of glucose and formation of lactic acid were followed during the incubation. Rose bengal was found to inhibit glucose disappearance in both non-photosensitized and photosensitized cells to the same degree. That glucose which disappeared in the rose bengal suspensions, either photosensitized or non-photosensitized was used as efficiently in producing lactic acid as was glucose in non-rose bengal treated controls. Increasing the suspension concentration of glucose removed the rose bengal inhibition. An analysis of the data by the Limeweaver-Burke equations indicated a competitive type of enzyme inhibition. It is concluded that one type of action rose bengal has on red cells is that of competing with glucose molecules for adsorption space on active hexokinase groups.

RICHARD P. BOYCE, JOHN D. SPIKES, and HENRY EYRING, University of Utah, Salt Lake City, Utah and U. S. Atomic Energy Commission, Washington, D. C. *Effects of formaldehyde on the mechanical properties of tendon.*

Effects of formalin solutions on load-strain curves, stress-relaxation curves, breaking strength and thermal contraction of rat tail tendon were studied as functions of concentration, pH, and temperature, respectively. Analysis of the temperature data was made by plotting the logarithm of the rate of relaxation at constant length versus the reciprocal of the absolute temperature. The resulting curves, and activation energies derived therefrom, were compared to those of untreated tendon. It is generally believed that at alkaline pH the ϵ-amino group of lysine in collagen reacts with formaldehyde to form part of a cross link. Increased cross-linkage should manifest itself in all of the mechanical properties studied above. Results show that such is the case. The effects appear to parallel to a certain degree the effect of aging.

RICHARD J. FREDERICK and DUGALD E. S. BROWN, University of Michigan, Ann Arbor, Michigan. *Tension in relation to pH, calcium and ouabain in glycerated muscle fiber systems.*

Glycerinated rabbit psoas muscle fibers have been prepared in which tension development is very sensitive to pH, Ca^{++}, and ouabain. Fibers were prepared in histidine-buffered (pH 6.8), 50% aqueous glycerol, and studied in a medium consisting of 10mM ATP, 4mM $MgCl_2$, and 125 mM Na maleate buffer. Four days after preparation, full tension in the fibers develops in less than 0.1 pH unit. After 10 days, full tension requires an increase of 0.2–0.3 pH unit. The fibers retain this pH sensitivity for two to three weeks. If CP is added to the medium a 0.8–1.0 pH unit increase is required to develop full tension. A relaxed fiber is brought to full tension by Ca^{++} in the range from 1–10 μM, but if CP is present, a range from 1–10mM is required. Ouabain, when added to the medium, also produces full tension in the range from 1–10 μg/ml provided the fiber is at its "trigger point." Equilibrating a relaxed fiber with 10 μg/ml ouabain greatly sensitizes it to pH, full tension then developing in less than 0.1 pH unit. pH sensitivity is similar to that shown by the untreated fibers studied 4 days after preparation. The relation between tension and concentration for these agents is analyzed and their effectiveness considered in terms of the number of active tension sites involved per mole of activator.

KARL F. GUTHE and JAMES A. WILSON, University of Michigan, Ann Arbor, Michigan. *Induction of contraction in glycerated muscle fiber systems by pH changes.*

Ralaxed rabbit psoas fibers, extracted in 50% glycerol, contract at a critical pH in a maleate-buffered solution containing Mg and ATP. Relaxed fibers were obtained by placing them in the ATP-medium at a pH below the contraction-inducing pH, whereupon they contract sharply and then immediately relax. A nearly isotonic recording lever was used to signal clearly the beginning of contraction as the pH was raised. The fiber goes from complete relaxation to full contraction in less than 0.2 pH unit. The pH at which contraction begins is sensitive to the duration of extraction in glycerol, to the temperature of extraction, to the temperature of the bathing solution, and to the ATP concentration. Enzymatic activity of fibers, under various conditions, has been measured in terms of inorganic phosphate released.

J. WOODLAND HASTINGS and VERNON C. BODE, University of Illinois, Urbana, Illinois. *Properties of the luminescent reaction in gonyaulax.*

Light emission in cell free extracts of the marine dinoflagellate *Gonyaulax polyedra* is dependent upon an enzyme (luciferase), a heat stable dialyzable substrate (luciferin) and relatively high salt concentrations. The total amount of light which has been obtained from extracts is only a small fraction of that which might have been obtained from the intact cells. A relatively rapid loss of activity is attributed to a dark destruction of luciferin, this reaction being favored in the presence of salt. Luciferin is bound by proteins which have specific binding properties, such as serum albumen, but not by other proteins. Bound luciferin is more stable and has a greater activity than free luciferin. These and other properties of luciferin will be discussed.

W. H. JOHNSON, University of Illinois, Urbana, Illinois. *The behavior of glycerinated muscle fibers containing paramyosin.*

The behavior of glycerinated skeletal muscles, such as the rabbit psoas, is well characterized. Many non-striated muscles contain large amounts of paramyosin, a protein which differs considerably from actomyosin, the main constituent of skeletal muscles. Paramyosin contains no ATP-ase but is characterized by the fact that it crystallizes out of solution very sharply as the pH is lowered. The actual value of pH lies in the range around 6.5 and depends on the ionic strength of the solution. Unloaded contractions of glycerinated muscle fibers depend in a similar manner on pH. We have examined the visco-elastic properties of such fibers as a function of pH by obtaining stress-strain curves after equilibration of fibers from the anterior byssus retractor muscle of *Mytilus edulis* in phosphate buffer containing 0.005 M mg++ at various pH and ionic strengths. If the fiber is first allowed to contract in 0.004 M ATP to a length below rest length (in the original muscle fiber), and is then washed in buffer, it is relatively stiff in the pH range where paramyosin crystallizes out of solution, while at higher pH, where paramyosin is soluble, the fiber is very plastic. Pyrophosphate (0.01 M), which renders psoas fibers plastic over a large pH range, produces little effect at low pH (ca. 6.0) on glycerinated Mytilus muscles, while at high pH (ca. 7.4) the fiber is more plastic in the presence of pyrophosphate. These results suggest that since paramyosin is present along with actomyosin in the myofilaments within these muscles, the two protein systems are mechanically in parallel, and that paramyosin under conditions where it is crystalline, could perhaps maintain tension or shortening developed by the actomyosin system.

REX B. SHAFER and JOHN D. SPIKES, University of Utah, Salt Lake City, Utah and United States Atomic Energy Commission, Washington, D. C. *Mechanical properties of tendons from rats deficient in calcium and vitamin D.*

It has been known for some time that the structure of many collagen-containing tissues is altered under conditions of malnutrition and disease. In the present investigation rats were raised on calcium and vitamin D deficient diets. The tail tendons were removed and subjected to mechanical analysis. An improved experimental setup was used to obtain load-strain and stress-relaxation curves directly by controlling the force parameter with a sensitive stretching machine and registering the force with the aid of a transducer and a Brown Recorder. This versatile apparatus also permitted breaking strengths and thermal contractions to be registered directly. The mechanical properties of the tendons which were used as a basis of study were the stress-relaxation behavior, load-strain behavior, breaking strength, thermal contraction temperature and the "critical" temperature. These investigations have shown that the dietary quantity of calcium or vitamin D has a definite effect on the mechanical properties of the tendon.

FRANK E. SOUTH, Jr., College of Medicine University of Illinois, Chicago, Illinois. *Hibernation, temperature and rates of oxidative phosphorylation by heart mitochondria.*[1]

The effect of temperature on rates of oxidative phosphorylation (pyruvate substrate) by heart mitochondria obtained from hibernating hamsters, control hamsters and rats was studied. Apparent energies of activation (*Ea*) determined between 5° and 24°C were, respectively, 20.4 20.8 and 28.3 k cal. for the rates of oxygen consumption and 20.6, 21.4 and 29.5 k cal. for the rates of phosphorylation. The differences between the rate and either groups of hamsters were significant statistically. While the slope of the regression line fitted to the data obtained hibernating animals did not differ significantly from that of the control hamsters, a parallel vertical displacement of the lines indicated a probable increase in these oxidative enzymes upon preparation for, or during, hibernation. No significant alterations in the efficiency of oxidative phosphorylation with variations in the incubation temperature were noted in any of the preparations. The results are implicated as a partial explanation of the ability of hibernants to maintain functional integrity at low body temperatures.

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LYELL J. THOMAS, Jr., University of Southern California, Los Angeles, California. *Calcium exchange in the frog's heart.*

Analyses of ashed muscle tissue show that the uptake of Ca^{45} by isolated frog heart ventricles perfused with normal Ringer's solution containing 1mM Ca^{++} is completed in about 30 minutes coinciding with the equilibration of $S^{35}O_4$ in the extracellular space. This Ca^{45} uptake probably occurs mainly by the exchange of labeled Ca^{+} for pre-existing surface bound Ca^{++}. The average amount of this normally exchangeable Ca^{++} in the muscle fibers is 0.8 mM/Kg wet weight and is not increased by longer perfusion (three hours), by more rapid rate of perfusion, or by rapid stimulation. However, the amount of labeled Ca^{++} taken up by ventricles perfused with K^{+}-free Ringer increases at a steady rate over a 60-minute period to twice the normal value coinciding with the gradual development of contracture and coinciding with an intracellular K^{+} loss and Na^{+} gain of about 25 mM/Kg. A comparison of simultaneous Ca^{45} and $S^{35}O_4$ washout rates from ventricles labeled in K^{+}-free Ringer to the washout rates from ventricles labeled in normal Ringer reveals that part of the extra Ca^{++} taken up during K^{+}-free perfusion penetrates into the muscle fiber surface. EDTA in K^{+}-free solution causes relaxation of contractured hearts and also greatly increases the permeability of the muscle fiber surface. These facts indicate that an excess of intracellular Ca^{++} is the cause of contracture resulting from K^{+}-free perfusion.

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